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DISPOSITION OF RADIOACTIVE 17α-HYDROXY-PROGESTERONE, 6α-METHYL-17α-ACETOXY-PROGESTERONE AND 6α-METHYLPREDNISOLONE IN HUMAN SUBJECTS*

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ABSTRACT

The disposition in the human body of the C^{14}-labeled synthetic steroids (6α-methyl-17α-acetoxyprogesterone, 6α-methylprednisolone and prednisolone) was compared to that of 3 natural steroids (cortisol, 17α-hydroxyprogesterone and progesterone). The unconjugated steroid metabolites of cortisol, 17α-hydroxyprogesterone, progesterone and 6α-methylprednisolone were cleared from the plasma at a rate with a half-life of approximately 1.5 hours. In contrast, prednisolone and 6α-methyl-17-acetoxyprogesterone were cleared at a much slower rate (half-life of over 200 minutes). Glucosiduronates of the progesterone group of compounds reached high levels in the plasma within fifteen minutes after intravenous injection, in contrast to the low and slowly achieved levels of glucosiduronates reached by the cortisol group of steroids. Other differences between the two groups were: higher theoretical volume of distribution shown by the former, higher urinary excretion of the latter, and substantial biliary and fecal excretion of the progesterone group of steroids. The differences in clinical effectiveness and physiologic effects of the various steroids are apparently not explicable in terms of single factors, but are probably due to a number of differences involving various characteristics of their metabolism, including differences in the clearance from plasma, in biliary excretion and in the routes of metabolism of the administered steroids.

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INTRODUCTION

MANY substituted steroids have been synthesized during recent years, but only a few have proved to be clinically more effective or to have different physiologic effects in man than the naturally occurring analogs. Little is known about the difference in metabolism between the naturally occurring steroids and the synthetic ones in the human subject. A few studies involving the comparative metabolism of the 2-methylated (1) or the Δ^1-analogs of cortisol or cortisone (2–5) have shown a slower rate of clearance of these compounds from the plasma of human subjects. Thus, in man, 2-methyl-cortisol appears to be metabolized differently than cortisol. The latter steroid is cleared more rapidly from the plasma than the former; the major portion of administered cortisol appears in the urine and plasma as glucosiduronates of the reduced steroids, whereas significant quantities of the metabolites of 2-methyl-cortisol have not been identified in the urine or plasma (1). The more pronounced clinical effectiveness of the Δ^1-steroids (*e.g.*, prednisolone), when compared on a dose basis to cortisol, was attributed to the slower metabolism of the unconjugated and unreduced "biologically active" steroid, indicating an inability of the body to metabolize Δ^1-steroids in the same manner or at the same rate as cortisol (2–4). Vermeulen (5) studied the biologic half-life in plasma of cortisol, prednisolone and dexamethasone and showed that the half-life of each of the two synthetic steroids was at least double that of cortisol. Other metabolic differences have been demonstrated between the Δ^1-steroids and cortisol—differences that involve decreases in conjugation and alteration in the reduction pattern (2, 4, 5).

MATERIAL AND METHODS

Steroids

The radioactive steroids investigated were 17α-hydroxyprogesterone-4-C^{14} (17-OH-Pg), 40 μc. per mg.; 6α-methyl-17α-acetoxyprogesterone-6-$C^{14}H_3$ (6α-CH_3-17α-AcOPg), 1.7 μc. per mg.; and 6α-methylprednisolone-6-$C^{14}H_3$ (6α-CH_3-prednisolone), 3.2 μc. per mg. The latter two steroids were synthesized in the laboratories of the Upjohn Company. All steroids were dissolved in ethanol immediately upon receipt and diluted with physiologic sodium chloride solution (1:30) just prior to intravenous injection. Paper chromatography of the steroids showed only traces (<1 per cent) of impurity. One subject received an intravenous infusion of 100 mg. of carrier 17α-hydroxyprogesterone dissolved in human serum albumin; at the end of the infusion the C^{14}-labeled steroid was administered through the tubing. The findings with the 3 steroids in this study were compared with previously published results obtained in our laboratory with 4-C^{14}-labeled cortisol, prednisolone and progesterone (3, 6).

Subjects

Subjects were either laboratory personnel or cancer patients without complicating liver or kidney disease (Table 1). Bile was collected from patients who had undergone cholecystectomy with insertion of a T-tube into the common bile duct. Complete bile

TABLE 1. SUMMARY OF CLINICAL DATA ON 19 SUBJECTS

Steroid injected	Subject			Clinical status	Dose injected (μc.)
	Name	Sex & age	Wt. (Kg.)		
17α-OH-Pg	J.Tr.	F 36	60	T-tube in common bile duct	3.0
17α-OH-Pg	T.G.	M 47	69	T-tube in common bile duct	4.5
17α-OH-Pg	J.S.	F 54	77.7	T-tube in common bile duct	2.0
17α-OH-Pg	M.F.	F 53	81	Ca. of breast	2.0
17α-OH-Pg	M.D.	F 65	66	Ca. of breast	2.0
17α-OH-Pg	J.Ta.	F 71	50	Ca. of breast	4.5*
6α-CH_3-17α-AcOPg	B.A.	F 48	65.6	Ca. of breast	2.4
6α-CH_3-17α-AcOPg	A.W.	F 53	52.2	Ca. of cervix	1.2
6α-CH_3-17α-AcOPg	G.C.	F 59	50.3	Ca. of cervix	1.2
6α-CH_3-17α-AcOPg	F.F.	F 85	43.6	Ca. of breast; generalized osteoporosis	1.2
6α-CH_3-17α-AcOPg	C.P.	M 19	84.1	Normal	1.2
6α-CH_3-17α-AcOPg	M.L.	M 25	77.7	Normal	1.2
6α-CH_3-17α-AcOPg	L.N.	M 33	78.6	Normal	1.2
6α-CH_3-prednisolone	A.M.	F 24	48	Osteoma of left femur	1.3
6α-CH_3-prednisolone	A.B.	F 35	54	Squamous-cell ca. of cervix	1.3
6α-CH_3-prednisolone	B.P.	F 39	60	Ca. of breast	1.3
6α-CH_3-prednisolone	L.H.	F 40	62.3	Weber-Christian disease; anemia	1.5
6α-CH_3-prednisolone	B.K.	F 48	60	Ca. of left breast	1.5
6α-CH_3-prednisolone	H.A.	F 64	73.3	Adenocarcinoma of uterus	1.3

* Plus 100 mg. of carrier.

collection was assured by the use of a suction apparatus attached to the T-tube, and confirmed by the appearance of acholic stools during the period of study. Liver function was normal preoperatively and the labeled steroids were administered three to four days postoperatively, after the condition of the patients had become stabilized without fever or other complications.

Methods

The techniques for hydrolysis, extraction and radioactive counting of the various steroids have been previously described (7a). For purposes of comparison and uniformity, it was assumed that each subject's blood volume constituted 9 per cent of his body weight, with 5 per cent as the plasma volume and 4 per cent as the total red blood cell mass.

Some aspects of the methods and terms used should be stressed. The radioactive steroids ($1.5–5.0 \times 10^6$ counts per minute) were injected intravenously in 30–50 ml. of physiologic saline over a period of two to three minutes. Blood samples (30–50 ml.) were withdrawn at indicated intervals. Hydrolysis with β-glucuronidase (Ketodase) was performed for seventy-two hours. On the basis of recently published evidence (7b), it may be assumed that this enzyme preparation hydrolyzed with ease the glucuronic acid conjugates of the steroids administered. The term "sulfates" refers to steroid metabolites which are recovered by continuous extraction with ether for forty-eight hours at pH 1.

RESULTS AND DISCUSSION

Blood

The data on the clearance of the various radioactive metabolites from the plasma and the radioactivity associated with the erythrocytes are

summarized in Table 2. A comparison is made in Figures 1 and 2 between the clearance from plasma of the unconjugated and conjugated metabolites of the 3 naturally occurring and the 3 synthetic steroids. The naturally occurring compounds (progesterone, 17α-hydroxyprogesterone and cortisol) had similar half-lives—99, 108 and 81 minutes, respectively. As shown previously (3), the additional double-bond of prednisolone markedly lengthened its half-life (210 minutes). The addition of a 6α-methyl group to prednisolone shortened its half-life considerably. That this is not a constant characteristic of 6α-methylation, however, is shown in the results for the 6α-methylated form of 17α-hydroxyprogesterone, even though the

TABLE 2. AMOUNT OF RADIOACTIVITY RELEASED FROM PLASMA AND ERYTHROCYTES BY SEQUENTIAL HYDROLYSIS AND EXTRACTION

Compound injected	No. of subjects	Per cent of injected dose in total plasma volume (±s.d.)				
		15 mins.	30 mins.	60 mins.	120 mins.	240 mins.
		Unconjugated				
17α-OH-Pg	6	4.5 ±2.1	2.7 ±1.1	1.5 ±0.5	0.85±0.33	0.48±0.17
6α-CH_3-17α-AcOPg	7	0.98±0.42	0.89±0.19	0.75±0.21	0.47±0.10	0.33±0.12
6α-CH_3-prednisolone	6	4.9 ±1.5	3.9 ±0.72	2.9 ±0.16	1.8 ±0.38	0.63±0.16
		Glucosiduronates				
17α-OH-Pg	6	25.3 ±6.8	24.1 ±3.9	20.9 ±5.1	16.9 ±5.2	11.8 ±3.8
6α-CH_3-17α-AcOPg	7	7.3 ±2.6	6.3 ±1.8	4.6 ±1.2	2.9 ±0.31	2.2 ±0.77
6α-CH_3-prednisolone	6	1.1 ±0.2	1.1 ±0.2	0.83±0.42	0.73±0.19	0.48±0.12
		"Sulfates"				
17α-OH-Pg	6	0.70±0.59	0.74±0.33	0.73±0.31	0.58±0.31	0.31±0.15
6α-CH_3-17α-AcOPg	7	0.12±0.07	0.42±0.33	0.26±0.10	0.29±0.19	0.26±0.16
6α-CH_3-prednisolone	6	0.42±0.32	0.45±0.22	0.55±0.24	0.55±0.23	0.37±0.15
		Strong acid hydrolysis				
17α-OH-Pg	6	pooled plasma 0.88±0.64				
6α-CH_3-17α-AcOPg	7	pooled plasma 0.06±0.03				
6α-CH_3-prednisolone	6	pooled plasma 0.33±0.18				
		Erythrocytes				
17α-OH-Pg	6	1.1±0.5	0.91±0.44	0.70±0.44	0.53±0.24	0.44±0.39
6α-OH-Pg	7	1.2±0.5	0.89±0.38	0.63±0.23	0.49±0.26	0.40±0.19
6α-CH_3-prednisolone	6	3.1±0.5	2.8 ±0.8	2.4 ±0.7	1.9 ±0.7	1.3 ±0.2

esterification of the tertiary hydroxyl group clouded the issue. In any event, methylprednisolone had a half-life (78 minutes) similar to that of cortisol, whereas 6α-methylacetoxyprogesterone had a half-life of 230 minutes. In addition, prednisolone, methylprednisolone and probably 6α-methylacetoxyprogesterone reached equilibrium very rapidly throughout the extravascular compartment, as demonstrated by the excellent fit of all points to a single straight line in the 3 cases. This is in contrast with the findings for the 3 natural steroids.

Examination of the plasma concentrations of the glucosiduronates, *i.e.*, the radioactivity released by β-glucuronidase hydrolysis, revealed that the levels of the progesterone-type compounds were substantially different from those of the cortisol-type steroids (Fig. 2). The levels of the former were remarkably high at fifteen minutes after injection and decreased

slowly, whereas the levels of the latter group were much lower. The concentrations of 17α-hydroxyprogesterone and progesterone metabolites were high in plasma at fifteen minutes (25 and 16 per cent of the injected dose, respectively) and they remained far above the levels of the glucosiduronates of the other steroids throughout the four-hour period of study. The con-

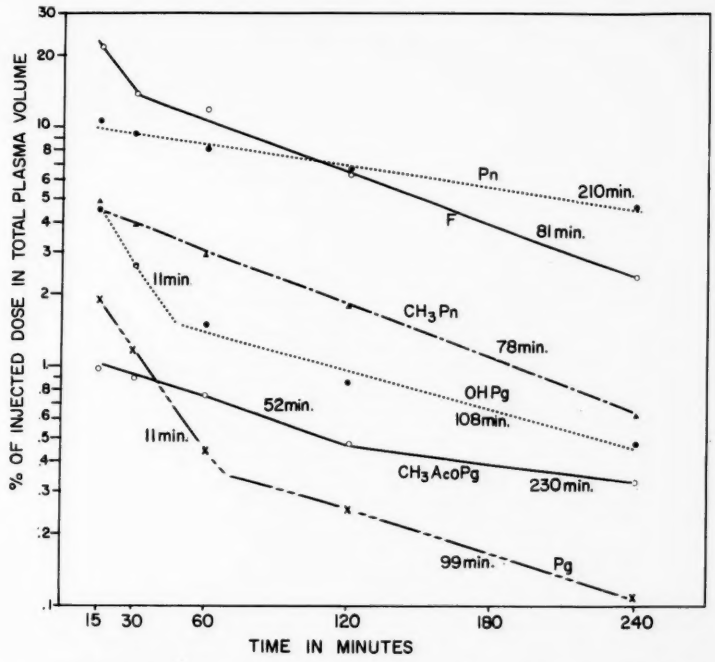

FIG. 1. The disappearance of unconjugated steroids from the plasma as a function time. F = cortisol; Pn = prednisolone; CH_3Pn = 6α-methylprednisolone; Pg = progesterone; OHPg = 17α-hydroxyprogesterone; CH_3AcOPg = 6α-methyl-17α-acetoxyprogesterone. The numbers with minutes refer to the T-$\frac{1}{2}$ of the various steroids. In the case of progesterone and 17-hydroxyprogesterone the T-$\frac{1}{2}$ for the initial rapid clearance rate (11 min.) is also shown.

centration of the glucosiduronates of 6α-methylacetoxyprogesterone metabolites was higher than that of the cortisol-like steroids at fifteen minutes (7 per cent), but was virtually the same as the concentration of the glucosiduronates of the cortisol metabolites which appeared at two hours. The high level of glucosiduronates of the progesterone-like compounds was probably a reflection of a very rapid reduction in ring A and conjugation coupled with a slow renal clearance of the conjugates. The lower concentration of glucosiduronates following administration of 6α-methyl-

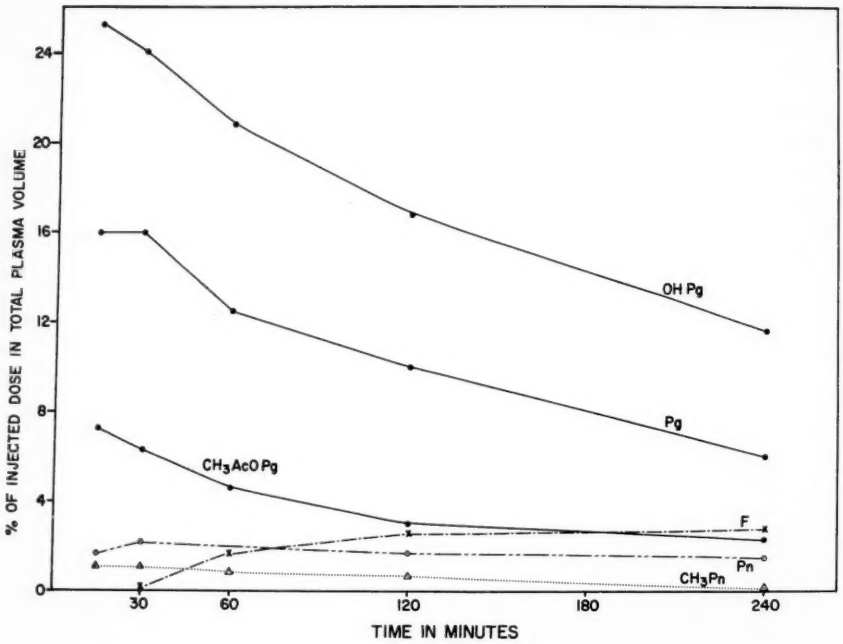

FIG. 2. The concentration of radioactive glucosiduronates (released by incubation with β-glucuronidase) in plasma as a function of time. Symbols are explained in the legend for Figure 1.

acetoxyprogesterone may have been a reflection of the slower metabolism of this steroid as compared to the other 2 progesterone-type steroids shown in Figure 2.

The relatively high percentage of radioactivity associated with the erythrocytes after the injection of C^{14}-6α-CH_3-prednisolone is noteworthy. It was the highest value observed for the 3 steroids presented in this paper and for those previously studied (3, 6). The relation of this finding to the physiologic effects of 6α-methylprednisolone deserves further study.

Urine and bile

The rates of urinary and biliary excretion of radioactivity are shown in Table 3. There was no apparent correlation between the rate of urinary excretion and the plasma concentration of unconjugated steroid or of metabolite glucosiduronates. The 2 compounds with the longest half-lives (prednisolone and 6α-methylacetoxyprogesterone) represent the extremes in urinary excretion, the former being the most rapidly excreted and the latter the most slowly excreted. Those compounds producing the highest

level of plasma glucosiduronates (17α-hydroxyprogesterone and progesterone) were excreted at an average rate.

The amount of radioactivity recovered in the urine and stool (Table 4) was correlated with the rate of urinary excretion, *i.e.*, the compounds that were excreted most slowly into the urine were associated with the lowest recovery of the radioactivity administered. The loss probably was related to the discontinuation of urine collections when the daily excretion became less than 1 per cent of the injected dose. Unpublished results from our laboratory indicate, for example, that following the intravenous administration of C^{14}-cholesterol, a subject may maintain a small but definite

TABLE 3. RATE OF URINARY AND BILIARY EXCRETION OF STEROIDS
(Values expressed as a percentage of the total radioactivity injected)

C^{14}-labeled steroid injected	Rate of excretion (% per hr.)						
	0–4 hrs.	4–8 hrs.	8–12 hrs.	12–24 hrs.	24–48 hrs.	48–72 hrs.	72–96 hrs.
Progesterone							
urine of T-tube pats. [4]*	3.5	2.1		0.85	0.23	0.17	0.10
urine of other pats. [5]	3.6	1.7		0.62	0.39	0.17	0.16
bile of T-tube pats. [4]	1.8	0.96		0.50	0.31	0.06	0.02
17α-Hydroxyprogesterone							
urine of T-tube pats. [3]	5.3	2.5	3.5	0.65	0.24	0.06	0.02
urine of other pats. [3]	5.8	2.9	1.7	0.86	0.27	0.07	0.04
bile of T-tube pats. [3]	1.7	0.93	0.52	0.23	0.05	0.04	0.01
6α-Methyl-17α-acetoxyprog.							
urine [7]	1.4	1.5	2.3	0.66	0.26	0.12	0.09
Cortisol							
urine [3]	7.1	8.0	4.5	1.0	0.18		
Prednisolone							
urine [3]	12.0	5.9	3.0	1.0	0.13		
6α-Methylprednisolone							
urine (6)	8.5	4.0	0.42	0.12	0.03		

* Values in brackets refer to the number of subjects.

excretion of radioactivity for more than a month. Thus, a trickle sustained for long periods of time may account eventually for all the radioactivity, especially if feces as well as urine are collected.

The values obtained for the urine and bile following the various hydrolytic procedures were in essential agreement with those of previous studies on related steroids (Fig. 3). For example, like cortisol and prednisolone, the metabolites of 6α-methylprednisolone were difficult to hydrolyze completely; moreover some may not have been extracted under the conditions used. Approximately half of the radioactivity initially present in the urine remained there after four consecutive hydrolytic and extractive procedures. This can be interpreted either as an unknown type of conjugation resistant to hydrolysis, or as the metabolism of 6α-methylprednisolone to very polar aglycones which could not be extracted with ether under the conditions of

our experiments. Similarly, 17α-hydroxyprogesterone closely resembled progesterone, in that the urinary metabolites were extensively hydrolyzed and extracted. β-Glucuronidase hydrolyzed the predominant portion of the conjugates. Bile, however, unlike the urine, contained no detectable unconjugated radioactive compounds, a low proportion of glucosiduronates and a large amount of labeled substances which either were not released

TABLE 4. RECOVERY OF INJECTED RADIOACTIVITY
(Values expressed as a percentage of the total radioactivity injected)

C^{14}-labeled steroid injected	Subject	Recovery (%)		
		urine	stool	total
17α-OH-Pg	J.Tr.	49	1	71*
	T.G.	64	0	72*
	J.S.	49	0	71*
	M.F.	69	2	71
	M.D.	57	18	75
	J.Ta.	57	10	67
	Average	58	10**	71
6α-CH_3-17α-AcOPg	B.A.	42	—	42
	A.W.	20	13	33
	G.C.	38	5	43
	F.F.	32	6	38
	M.L.	37	—	37
	L.N.	28	—	28
	Average	33	8	38
6α-CH_3-prednisolone	A.M.	63	6	69
	A.B.	79	13	92
	B.P.	87	11	98
	L.H.	57	5	62
	B.K.	83	10	93
	H.A.	78	11	89
	Average	75	9	84

* Biliary excretion values for J.Tr., T.G. and J.S. were 21, 8 and 22 per cent respectively.
** Average for subjects without biliary fistulas.

by hydrolysis or were difficult to extract. Results with methylacetoxyprogesterone closely resembled those with 17α-hydroxyprogesterone.

From the extrapolation of the clearance curves of the unconjugated injected C^{14}-steroids to zero time, it is possible to calculate the theoretical volume of distribution of a given steroid in the body (8). On the basis of the data presented (Figs. 1 and 2, Table 5), it appears that the smallest volume of distribution was associated with injected C^{14}-cortisol. Thus at zero time about 15–20 per cent of the C^{14}-cortisol was present in the total plasma volume, compared to 10 per cent for C^{14}-prednisolone and 5 per cent for C^{14}-6α-methylprednisolone. Since the remaining percentage of the steroids, not present in the plasma, would be distributed in a theoretical

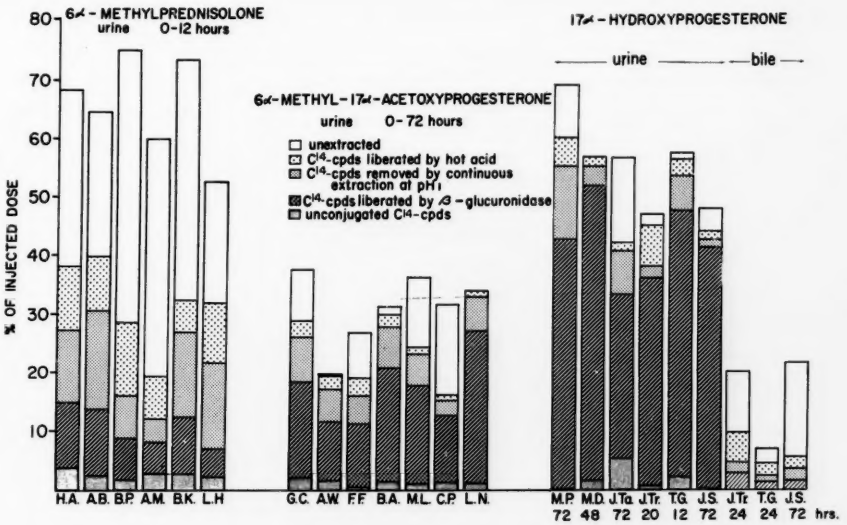

FIG. 3. Hydrolysis of urinary and biliary C^{14}-metabolites. The times (hrs.) below the abscissa in the 17α-hydroxyprogesterone group refer to the duration of collection. In the case of 6α-methylprednisolone, only 0–12 hour samples were used for hydrolysis.

volume which would exceed that of the total body water, the data point to concentration of the steroids in some special body tissue or compartment. The presence of a fairly large proportion (5–20 per cent) of the injected radioactivity in the plasma following the injection of the cortisol-like compounds, may have been related to their attachment to transcortin, a plasma protein with high affinity for cortisol and related compounds (9). It is of interest that 17-α-hydroxyprogesterone, which has considerable affinity for transcortin (10), was present at zero time in the

TABLE 5. DISTRIBUTION OF RADIOACTIVITY IN PLASMA, URINE, BILE AND STOOLS FOLLOWING INTRAVENOUS INJECTION OF C^{14}-STEROIDS*

C^{14}-steroid injected	Per cent of injected steroid in total plasma volume			Per cent of injected radioactivity excreted in						
				Urine					Bile (total)	Stool** (total)
	unconjugated at 0 time	glucosiduronates		total	unconjugated	glucosiduronates	"sulfates"	acid hydrolysis		
		15 mins.	4 hrs.							
Cortisol	15–20	<1	3–4	>90	< 2	40–50	10	5	<5	1–2
Prednisolone	10	2	2	>90	>10	40–50	5–10	1–2	<5	1–2
6α-Methylprednisolone	5	1	<0.5	75	2.5	<10	10	10	20	9
Progesterone	0.6	16	6	52	1–2	40	6	5	30	13
17-OH-Pg	2	25	12	58	<2	40–50	5–10	1–3	15–20	10
6α-Methyl-17-AcOPg	0.8	7	3	33	1	15–20	5	2–3	35–40	>8

* A composite of results published in the literature and of unpublished data from our laboratory. The percentages represent the approximate means of the various values.

** Values obtained in subjects without bile fistulas. In patients with bile fistulas the values for the stools usually were less than 1 per cent.

highest concentration of any of the progesterone-like compounds. Thus, it appears that the affinity of some tissues for the progesterone group of compounds is higher than that exhibited by the plasma proteins, with resulting rapid diffusion of the steroids from the blood. In the case of progesterone-like compounds, storage in the fat depots of the body may play an important role (11); with the other steroids the liver seems to be of paramount importance in the concentration and removal of the radioactive steroids from the plasma (5, 12).

The data presented in Table 5 indicate that increased effectiveness of a steroid in relation to weight (*e.g.*, 6α-methylprednisolone) may not necessarily be associated with decreased clearance, and hence metabolism, as has been postulated for prednisolone (3, 4). The effectiveness of 6α-methylprednisolone may be related to a different type of metabolism as shown by the small amount of hydrolyzable and/or extractable metabolites when compared to those of cortisol and prednisolone. The radioactivity remaining in the urine after hydrolysis and extraction of 6α-methylprednisolone may be due either to unusual conjugates or to metabolites of a very hydrophilic nature. Differences from cortisol and prednisolone are also demonstrated by the substantial excretion of 6α-methylprednisolone metabolites in the bile and stools. On the other hand, the greater and more prolonged progestational effect of 6α-methyl-17α-AcOPg and its effectiveness by the oral route (13) may be related to its slower metabolism when compared to that of progesterone. 6α-Methyl-17α-AcOPg apparently is stored in some compartment of the body for a much longer period of time than are any of the steroids studied, as indicated by the low excretion of radioactivity in the urine. In other respects the progestational group of hormones showed similar metabolic rates and distributions.

The urinary excretion of substantial amounts of unconjugated and unchanged steroid was characteristic only of prednisolone. This finding has been substantiated in several other studies (5, 14, 15), and is also true for other Δ^1-steroids, such as dexamethasone and triamcinolone (5). The biliary excretion of the progesterone-like compounds was substantial, especially that of progesterone and 6α-methyl-17α-AcOPg, in contrast to the relatively low biliary excretion of the cortisol-like steroids.

Thus, from the data presented, it would appear that the more pronounced clinical effectiveness of some of the synthetic steroids (prednisolone, 6α-methylprednisolone and 6α-methyl-17α-acetoxyprogesterone) cannot be explained on the basis of a single metabolic phenomenon. There are differences: 1) in the urinary excretion of unconjugated steroids; 2) in plasma and urinary clearance rates; 3) in the associations with plasma protein and the erythrocytes; and 4) in the amounts excreted in the bile or stools.

These differences point to a number of metabolic characteristics that may involve the steroid in question, and may also influence and direct its effects on body metabolism.

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STUDY OF 17-HYDROXYPROGESTERONE-4-C^{14} IN MAN*

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ABSTRACT

The peripheral metabolism of 17-hydroxyprogesterone-4-C^{14} was studied in a normal subject and a patient with untreated congenital adrenal hyperplasia, to examine the influence of small and large endogenous production of this hormone on the fate of the tracer. The excretion of radioactivity was slow in both subjects. Pregnane-3α,17α,20α-triol and 3α,17α-dihydroxypregnane-20-one were the major metabolites, whereas their epimers accounted for only a small percentage of the total radioactivity. Formation of androsterone and etiocholanolone occurred to a limited extent; this metabolic transformation in the patient with congenital adrenal hyperplasia was found to be double that in the normal subject.

THE excessive urinary excretion of pregnane-3α,17α,20α-triol by patients with congenital adrenal hyperplasia (CAH)[1] is considered to be one of the characteristics of the common form of the disorder (1). This metabolite has been found to be derived from 17-hydroxyprogesterone in isolation studies following administration of large amounts of nonisotopic hormone (1–5). These studies have shown in addition that 17-hydroxyprogesterone is metabolized to the epimers of pregnanetriol, to 17-hydroxypregnanolone and its 5α-H epimer, and to the 17-ketosteroids, androsterone and etiocholanolone (2–6). The present investigation with 17-hydroxyprogesterone-4-C^{14} was undertaken to establish a firmer basis for the precursor-metabolite relationship, both qualitatively and quantitatively. The metabolic behavior of 17-hydroxyprogesterone-4-C^{14} in a normal and a CAH subject was examined in detail since in this way the influence of large and

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[1] The following abbreviations and trivial names are used in this report: CAH, congenital adrenal hyperplasia; 17-hydroxyprogesterone, 17α-hydroxy-Δ^4-pregnene-3,20-dione; pregnanetriol, pregnane-3α,17α,20α-triol; 17-hydroxypregnanolone, 3α,17α-dihydroxypregnane-20-one; androsterone, 3α-hydroxyandrostane-17-one; etiocholanolone, 3α-hydroxyetiocholane-17-one.

small endogenous production on the fate of the tracer could be studied. It was also possible to estimate the glandular production of 17-hydroxyprogesterone and the relative contribution of this hormone to the endogenous 17-ketosteroids, androsterone and etiocholanolone.

It should be emphasized that this investigation was designed primarily to study the metabolic fate of 17-hydroxyprogesterone in the presence and in the absence of a large endogenous production of this hormone. It was not intended as a comparison of the normal person with the CAH patient since this would be unwarranted with only one representative of each condition. It is, however, justifiable to point out certain consequences

TABLE 1. URINARY EXCRETION OF RADIOACTIVITY FOLLOWING ADMINISTRATION OF 17-HYDROXYPROGESTERONE-4-C^{14}

	Urine (10^5 c.p.m.)	Neutral extract* (10^5 c.p.m.)
Subject M		
Dose administered, 35.8×10^5 c.p.m.		
Day 1	15.8	15.3
2	4.48	3.90
3	1.18	1.01
4	0.40	
5	0.25	
Subject R		
Dose administered, 39.0×10^5 c.p.m.		
Day 1	15.9	14.3
2	3.74	3.62
3	2.00	1.60†

* Ketodase hydrolysis.
† Ketodase hydrolysis followed by continuous ether extraction at 1 *N* acid.

which are evident from the great overproduction of steroid in CAH, in connection with the basic studies on the metabolism of 17-hydroxyprogesterone.

METHODS

Patient M, a 61-year-old man with multiple sclerosis, was normal with respect to his adrenal and hepatic function. Patient R, aged 42, had congenital adrenal hyperplasia and was untreated for this disorder.

Each subject received approximately 0.1 mg. of 17-hydroxyprogesterone-4-C^{14} (32 μc. per mg., in 1 ml. of propylene glycol), injected into the polyethylene tubing leading from a 5 per cent glucose solution into the cubital vein. The syringe and needle containing the radioactive solution were weighed before and after injection, for measurement of the dose (Tables 1 and 2).

The urine from Patient M was collected for five days following administration of the hormone and the radioactivity present was counted (Table 1) by the methods (7) previously reported. The first day's urine was collected at hourly intervals during the first six hours, followed successively by a six-hour and a twelve-hour period. The cumulative urinary excretion of radioactivity is shown in Figure 1. The various urine samples of the first day were combined for hydrolysis. The first three-day urine collections were sep-

arately incubated with β-glucuronidase,[2] and the neutral fractions were prepared by the usual methods employed in these laboratories (8). The radioactivity found in these extracts is recorded in Table 1. Blood samples (30 ml.) were collected thirty minutes and two hours after administration of the hormone and processed by addition of 300 ml. of 1:1 acetone-ethanol mixture. The precipitates were subjected to thorough extraction with 300 ml. of the same mixture. Radioactivity was 175,000 c.p.m. per liter of blood in the soluble extract for the thirty-minute sample, and 170,000 c.p.m. for the two-hour sample. Feces from Patient M were collected for three days; these contained radioactivity equivalent to 20 per cent of that administered. Patient R refused to collect these specimens.

The urine from Patient R was collected for three days following administration of

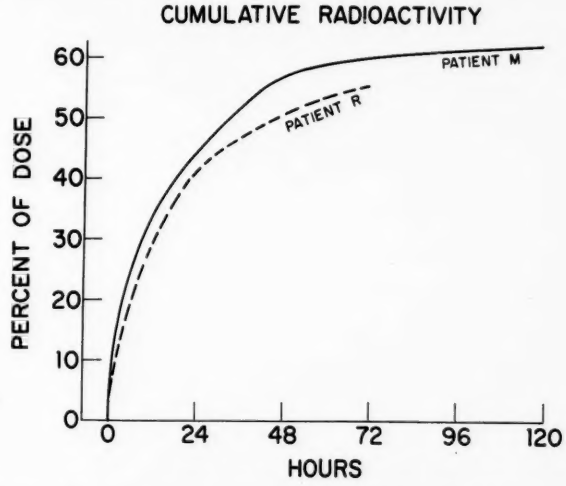

FIG. 1. Cumulative urinary excretion of radioactivity following intravenous administration of 17-hydroxyprogesterone-4-C^{14} in Patients R and M.

the dose (Table 2); the cumulative urinary excretion of radioactivity is shown in Figure 1. The conjugates were hydrolyzed by β-glucuronidase and the neutral steroid fraction prepared (Table 1). The hydrolysis with enzyme was less complete on the third day. Therefore, in this instance the residual urine and the aqueous and alkaline washes of this day were combined, acidified to 1 *N* with sulfuric acid and continuously subjected to extraction with ether for forty-eight hours. The neutral fraction was combined with the neutral steroid fraction obtained after enzyme hydrolysis.

Metabolites of 17-hydroxyprogesterone-4-C^{14}

Patient M: Four portions of the neutral steroid extract of the first day's urine were diluted with nonisotopic carrier steroids. To one was added pregnane-3α,17α,20α-triol. To the second were added pregnane-3α,17α,20β-triol and 3α,17α-dihydroxypregnane-20-one. To the third were added allopregnane-3α,17α,20α-triol and 3α,17α-dihydroxy-

[2] β-Glucuronidase, known as Ketodase, was obtained from the Warner-Chilcott Laboratories, a division of Warner-Lambert Pharmaceutical Company, New York, N. Y.

TABLE 2. ANALYSIS OF THE METABOLITES OF 17-HYDROXYPROGESTERONE-4-C^{14} BY REVERSE ISOTOPE DILUTION

(Patient M)

	Neutral steroid extract used (10^3 c.p.m.)	Carrier steroid (mg.)	Specific activity (c.p.m./mg.)	Total activity (10^3 c.p.m.)	Per cent of neutral extract
Day 1					
Pregnane-3α,17α,20α-triol	91.8	30.1	974	29.2	31.8
Pregnane-3α,17α,20β-triol	153	14.7	222	3.3	2.1
3α,17α-Dihydroxypregnane-20-one		27.4	2,850	78.0	51.0
Allopregnane-3α,17α,20α-triol	153	30.0	31	0.9	0.6
3α,17α-Dihydroxyallopregnane-20-one		30.0	114	3.4	2.2
3α-Hydroxyandrostane-17-one	153	34.9	18	0.6	0.4
3α-Hydroxyetiocholane-17-one		30.3	65	2.3	1.5
Day 2					
Pregnane-3α,17α,20α-triol	39.0	34.6	700	24.2	62.0
3α,17α-Dihydroxypregnane-20-one		28.4	166	47	12.0

allopregnane-20-one. To the fourth were added 3α-hydroxyandrostane-17-one and 3α-hydroxyetiocholane-17-one. The first three mixtures containing the C-21 metabolites were chromatographed on a partition column containing 40 Gm. of silica gel and 16 ml. of ethanol. The columns were developed in essentially the same manner, starting with 1 per cent ethanol in methylene chloride. 3α,17α-Dihydroxyallopregnane-20-one was eluted with 2 per cent ethanol in methylene chloride and its 5β epimer was eluted with 3 per cent ethanol in methylene chloride. All three triols were eluted with 5 per cent ethanol in methylene chloride. The fourth mixture containing the C-19 ketosteroids was also chromatographed on 40 Gm. of silica gel containing 16 ml. of ethanol. The two C-5 epimers were separated by elution with 1 per cent ethanol in methylene chloride-petroleum ether (1:3). The steroids were then recrystallized to constant specific activity. When there was some uncertainty about the constancy of the specific activity, the steroid was acetylated and recrystallized. The portion used, the carrier added and the specific and total activity obtained are recorded in Table 2.

TABLE 3. ANALYSIS OF THE METABOLITES OF 17-HYDROXYPROGESTERONE-4-C^{14} BY PAPER CHROMATOGRAPHY*

Distance from origin (inches)	Reference steroid	Per cent of urinary neutral steroid extract			
		Patient M			Patient R
		Day 1**	Day 2§	Day 3‖	Day 1¶
Origin–2		1.9	5.6	4.2	8.1
2–16		3.4		4.2	
16–20	Pregnane-3α,17α,20α-triol† / Allopregnane-3α,17α,20α-triol†	34.2‡	69.0‡ (64† / 2.2†)	70.0‡	37.8‡
20–22	Pregnane-3α,17α,20β-triol	2.0	1.8	1.3	2.7
22–28		0.8	2.2		
28–32½	3α,17α-Dihydroxypregnane-20-one	50.5	12.8	9.4	37.1
32½–38		1.1	1.9	1.3	4.6‡
38–42	3α-Hydroxyandrostane-17-one / 3α-Hydroxyetiocholane-17-one	1.9‡	5.4‡	9.9‡	
42–end, and effluent		0.7	0.6	1.2	0.2

* The solvent system was isooctane:toluene:methanol:water (3:5:4:1) at 23° C. for 28 hours.
** 91,800 c.p.m. used.
† These epimeric triols could be separated by developing the paper for 40 hours instead of 28 hours.
‡ These values represent the radioactivity present in the area of the paper where the reference steroids move.
§ 39,000 c.p.m. used.
‖ 20,200 c.p.m. used.
¶ 28,600 c.p.m. used.

TABLE 4. COMPARISON OF RESULTS OBTAINED BY REVERSE ISOTOPE DILUTION AND PAPER CHROMATOGRAPHY

(*Patient M*)

Steroid	R.I.D.*	P.C.**
Day 1		
Pregnane-3α,17α,20α-triol	31.8	34.2
Allopregnane-3α,17α,20α-triol	0.6	
Pregnane-3α,17α,20β-triol	2.1	2.0
3α,17α-Dihydroxypregnane-20-one	51.0 (49.0)†	50.5
3α,17α-Dihydroxyallopregnane-20-one	2.2	
3α-Hydroxyandrostane-17-one	0.4	1.9
3α-Hydroxyetiocholane-17-one	1.5	
Day 2		
Pregnane-3α,17α,20α-triol	62.0	64.0
Allopregnane-3α,17α,20α-triol		2.2
3α,17α-Dihydroxypregnane-20-one	12.0 (11.8)†	12.8
Day 3		
Pregnane-3α,17α,20α-triol	(69.3)†	70.0
3α,17α-Dihydroxypregnane-20-one	(6.4)†	9.4

* R.I.D. = reverse isotope dilution.
** P.C. = paper chromatography.
† R.I.D. of P.C. eluate.

The second day's extract was worked up in the same manner for pregnanetriol and 17-hydroxypregnanolone. The results are recorded in Table 2.

The analyses by paper chromatography were performed in a cylindrical glass tank (4 feet high and 12 inches in diameter) with Whatman No. 1 paper (47×7 inches) as described earlier (7). The solvent system employed was isooctane:toluene:methanol:water (3:5:4:1). The paper was developed for twenty-eight hours at 23° C. The radioactive metabolites were located by use of an Aquebogue scanner and by reference steroids chromatographed simultaneously. The areas of radioactivity were eluted with chloroform-methanol (1:1). The results obtained from the analysis of the neutral extracts of Days 1, 2 and 3 are recorded in Table 3. Reverse isotope dilution analysis was also carried out on certain eluates from paper by addition of weighed amounts of carrier, and recrystallization to constant specific activity. These results are shown in Table 4.

Patient R: Reverse isotope dilution studies were made on a portion of the neutral steroid extract of the urine obtained on the first and second day following the tracer

TABLE 5. ANALYSIS OF THE METABOLITES OF 17-HYDROXYPROGESTERONE-4-C^{14} BY REVERSE ISOTOPIC DILUTION

(*Patient R*)

	Neutral steroid extract used (10^3 c.p.m.)	Carrier steroid (mg.)	Specific activity (c.p.m./mg.)	Total activity (10^3 c.p.m.)
Day 1	28.3			
Pregnane-3α,17α,20α-triol		33.3	299	10,000
3α,17α-Dihydroxypregnane-20-one		32.5	296	9,600
Day 2	36.0			
Pregnane-3α,17α,20α-triol		34.9	287	11,300*
3α,17α-Dihydroxypregnane-20-one		30.5	75	2,500†

* Calculation includes 45 mg./day of endogenous metabolite.
† Calculation includes 32 mg./day of endogenous metabolite.

dose of 17-hydroxyprogesterone. Carrier pregnane-3α,17α,20α-triol and 3α,17α-dihydroxypregnane-20-one were added to the aliquot and chromatographed on silica gel as in the other subject. The amounts used, the carrier added and the specific and total activity obtained are recorded in Table 5.

Paper chromatographic analysis was employed for the neutral extracts of each day. The results for pregnane-3α,17α,20α-triol and 3α,17α-dihydroxypregnane-20-one on the first day were similar to those from reverse isotope dilution studies (Table 3). The chromatographic separation of the neutral extracts of the second and third day was poor because of the unusually large amounts of steroid in the neutral extracts and the low activity.

Specific activity studies: The specific activity of the carrier-free metabolites of 17-hydroxyprogesterone-4-C^{14} present in the neutral steroid extracts was studied in Patient R. Eighty per cent (1.14×10^6 c.p.m.) of the neutral extract of Day 1 was chromatographed on 80 Gm. of silica gel containing 32 ml. of ethanol. Elution with 500 ml. of 1 per cent ethanol in methylene chloride and 250 ml. each of 2 per cent and 3 per cent ethanol in methylene chloride yielded fractions (7.73×10^4 c.p.m.) containing androsterone and etiocholanolone. Further elution with 3 per cent ethanol in methylene

TABLE 6. SPECIFIC ACTIVITIES OF 17-HYDROXYPROGESTERONE-4-C^{14} METABOLITES IN PATIENT R

	Specific activity (c.p.m./μg.)			
	Day 1	Day 2	Day 3	Average
Pregnane-3α,17α,20α-triol	11.5	2.41	0.32	4.70
3α,17α-Dihydroxypregnane-20-one	16.2	0.71	0.18	5.40
3α-Hydroxyandrostane-17-one	2.33	2.09	1.33	1.91
3α-Hydroxyetiocholane-17-one	3.50	2.56	2.33	2.79

chloride at a rate of 10 ml. per thirty minutes afforded 31 mg. of 17-hydroxypregnanolone. Recrystallization from ethyl acetate and methanol followed by acetylation and recrystallization from ethyl acetate gave a constant specific activity of 16,200 c.p.m. per mg. for 17-hydroxypregnanolone.

Elution with 6 per cent ethanol in methylene chloride afforded 40 mg. of pregnane-3α,17α,20α-triol. Recrystallization from methanol followed by acetylation and recrystallization of the diacetate from methanol yielded pregnanetriol at a constant specific activity of 11,500 c.p.m. per mg. Chromatography of this triol on Whatman No. 1 paper in the system isooctane:toluene:methanol:water (3:5:4:1) demonstrated that it was uncontaminated with its isomers.

The fractions containing androsterone and etiocholanolone were combined and chromatographed on 80 Gm. of silica gel containing 32 ml. of ethanol. Separation was achieved by elution with 1 per cent ethanol in petroleum ether-methylene chloride (7:3). Each steroid was further purified by chromatography on Whatman No. 1 paper in the system isooctane:methanol:water (10:10:1) for eighteen hours at 23°C. The specific activity was obtained by analysis for radioactivity and by the Zimmermann reaction. The results are recorded in Table 6.

The specific activities for these 4 compounds in the neutral extracts of Days 2 and 3 were similarly obtained and the results are recorded in Table 6. Only 70 per cent of the neutral extract of these days was used—2.54×10^5 c.p.m. and 1.12×10^5 c.p.m. respectively. The less polar fractions from the first chromatographic column containing

androsterone and etiocholanolone contained 8.87×10^4 c.p.m. and 3.41×10^4 c.p.m. for Days 2 and 3 respectively. Androsterone and etiocholanolone isolated from the chromatograms of the neutral extract of Day 2 were also crystallized. The specific activity, determined by weighing the steroids and analyzing for radioactivity, was identical with that obtained when the Zimmermann reaction was employed for measurement of the steroids.

Endogenous steroids: The endogenous production of the metabolites of 17-hydroxyprogesterone by Patients M and R is shown in Table 9. The methods for the ketosteroids have been reported previously (8, 9). The amounts of pregnanetriol and 17-hydroxypregnanolone were estimated by the 3α-hydroxy dehydrogenase preparation of Hurlock and Talalay (10).

RESULTS

In contrast to the other adrenocortical steroids previously studied (11–13), the rate of urinary excretion of radioactivity was slow following intravenous administration of 17-hydroxyprogesterone-4-C^{14} in both patients (Fig. 1). There was still a measurable amount of radioactivity in the urine on the fifth day (Table 1). However, the major portion of the radioactivity (55 per cent of the dose) was eliminated during the first three days. The urinary conjugates were readily and essentially completely hydrolyzed in both subjects by β-glucuronidase followed by continuous ether extraction at pH 1 (Table 1).

Five C_{21}-metabolites and two 17-ketosteroids together accounted for 90 per cent of the total radioactivity[3] present in the first day's urine of Patient M. 17-Hydroxypregnanolone was the principal component (51 per cent) and pregnane-3α,17α,20α-triol was present in slightly smaller amount (32 per cent). The epimers of these compounds, *i.e.*, 3α,17α-dihydroxyallopregnane-20-one and pregnane-3α,17α,20β-triol, were formed in equal amount and together represented somewhat more than 4 per cent of the total radioactivity; the amount of allopregnane-3α,17α,20α-triol was almost negligible. About 2 per cent of the radioactivity in the neutral steroid fraction of the first day was divided between androsterone and etiocholanolone. Thus, although there is a path for metabolic removal of two carbons of the side-chain, this is a less favored route of transformation.

The emphasis in Patient M during the second and third day was placed on pregnane-3α,17α,20α-triol and 17-hydroxypregnanolone. The amount of 17-hydroxypregnanolone decreased sharply after the first day from 51 per cent of the neutral extract to 6 per cent on the third day (Table 7). Pregnane-3α,17α,20α-triol, however, was the major component found during the second and third day; it accounted for over 60 per cent of the total radioactivity.

[3] Total radioactivity refers to the neutral steroid extract obtained from the 24-hour urine collection. Since the hydrolysis of the conjugates was essentially quantitative, the value is to all purposes equivalent to the amount in the urine.

TABLE 7. C_{21} METABOLITES OF 17-HYDROXYPROGESTERONE-4-C^{14}

	Day 1		Day 2		Day 3		Total	
	PT*	17-OHP**	PT	17-OHP	PT	17-OHP	PT	17-OHP
Patient M								
Per cent of neutral steroids	31.8	51.0	62.0	12.0	69.3	6.4	39.5	41.2
Per cent of dose	13.6	21.8	6.8	1.3	2.0	0.2	22.3	23.3
Patient R								
Per cent of neutral steroids	35.3	34.0	31.2	6.9	9.0	3.8	32.3	26.6
Per cent of dose	12.9	12.5	2.9	0.6	0.4	0.2	16.2	13.3

* Pregnane-3α,17α,20α-triol.
** 3α,17α-Dihydroxypregnane-20-one.

Since it was clear from these results that only 2 of the C_{21}-metabolites were important in a quantitative sense, pregnane-3α,17α,20α-triol and 17-hydroxypregnanolone were examined in Patient R. These were found in equal amounts on the first day in Patient R (Table 7), whereas the amount of 17-hydroxypregnanolone was almost twice that of pregnanetriol on the first day in Patient M. The output of 17-hydroxypregnanolone decreased sharply thereafter as in the other subject. Pregnanetriol production decreased in Patient R during the second day and there was an acute decline on the third day. The triol thus comprised a smaller fraction of the dose as well as of the total radioactivity on these days than on the first day. Over the three-day period, 17-hydroxyprogesterone was metabolized equally to pregnanetriol and 17-hydroxypregnanolone in Patient M, whereas the triol was produced in larger amount than the 20-ketosteroid in Patient R. It is noteworthy that the ratio of the radioactivity of these 2 steroids was similar to the ratio of the same 2 steroids produced endogenously by Patient R.

The transformation of 17-hydroxyprogesterone to androsterone and etiocholanolone by both subjects was small (Table 8); etiocholanolone was

TABLE 8. 17-KETOSTEROID METABOLITES OF 17-HYDROXYPROGESTERONE-4-C^{14}

	Day 1		Day 2		Day 3		Total	
	A*	E**	A	E	A	E	A	E
Patient M								
Per cent of neutral steroids	0.4	1.5	5.4		9.9		2.9	
Per cent of dose	0.2	0.6	0.6		0.3		1.7	
Patient R								
Per cent of neutral steroids	0.9	2.6	3.3	7.5	4.7	15.5	1.7	4.6
Per cent of dose	0.3	0.9	0.3	0.7	0.2	0.6	0.8	2.3

* 3α-Hydroxyandrostane-17-one.
** 3α-Hydroxyetiocholane-17-one.

the major component of the pair of metabolites, as earlier reported (3, 4, 6). In both patients these 17-ketosteroids represented a larger portion of the urinary radioactivity of the second and third days after the administration of tracer. This was more evident with Patient R. In fact, with this patient, etiocholanolone alone (15 per cent) was quantitatively a more important metabolite on the third day than pregnanetriol and 17-hydroxypregnanolone combined (12.8 per cent). In this respect the metabolism of 17-hydroxyprogesterone differed considerably between the 2 subjects. In Patient M the 2 major C_{21}-metabolites studied accounted for at least 75 per cent of the daily total radioactivity, whereas in Patient R the radioactivity attributable to these compounds decreased with time from 69 to 13 per cent. The radioactivity due to androsterone and etiocholanolone, on the other hand, increased progressively from 4 to 20 per cent of the total during the three successive days.

TABLE 9. DAILY ENDOGENOUS PRODUCTION

	Patient M	Patient R
3α-Hydroxyandrostane-17-one	2.2 mg.	5.7 mg.
3α-Hydroxyetiocholane-17-one	2.2 mg.	10.6 mg.
Pregnane-3α,17α,20α-triol	0.9 mg.	45.0 mg.
3α,17α-Dihydroxypregnane-20-one	—	32.0 mg.

The quantitation of the radioactive metabolites of 17-hydroxyprogesterone was also made by paper chromatography (Tables 3 and 4). The results thus obtained for pregnanetriol, 17-hydroxypregnanolone, androsterone and etiocholanolone were in agreement with those achieved by reverse isotope dilution. The remainder of the radioactivity (Table 3) was distributed in compounds of similar polarity, and about 5 to 8 per cent was in substances with mobilities slower than pregnanetriol.

Specific activities. It was possible to measure the specific activity of pregnanetriol, 17-hydroxypregnanolone, androsterone and etiocholanolone each day in Patient R (Table 6). The high specific activity of 17-hydroxypregnanolone (16.2 c.p.m. per μg.) on the first day together with the subsequent prompt fall were related to the initial lower level of pregnanetriol (11.5 c.p.m. per μg.) and its slower decline. The results were consistent with the conclusion that 17-hydroxypregnanolone is the precursor of pregnanetriol. The specific activities of androsterone and etiocholanolone were much lower (2.33 and 3.50 c.p.m. per μg. respectively) than those of the C_{21}-metabolites on the first day and, in contrast with the pregnane metabolites, the specific activity of 17-ketosteroid decreased slowly. The specific activity of each of the 4 metabolites differed from the others, whether this value was calculated for the whole collection period of the study or for the

individual days. A more extended urine collection might have permitted the specific activities of pregnanetriol and 17-hydroxypregnanolone to approach each other. However, it is scarcely possible that androsterone and etiocholanolone could have attained the specific activity of the C_{21}-metabolites. These results afford conclusive evidence for the formation of these two 17-ketosteroid metabolites from hormones other than 17-hydroxyprogesterone. The slower decline in specific activity of androsterone and etiocholanolone in contrast to the precipitous drop in specific activity of pregnanetriol and 17-hydroxypregnanolone suggests that the 17-ketosteroids would still retain significant radioactivity for several days beyond the collection period studied, and at a time when pregnanetriol and 17-hydroxypregnanolone would have virtually no activity.

DISCUSSION

One of the most interesting results derived from these studies is an estimate of the daily secretion of 17-hydroxyprogesterone both by the normal subject and the patient with adrenal hyperplasia. This calculation can be made either from the known endogenous production of the pertinent metabolites in terms of the fraction of the administered radioactivity recovered as that metabolite (11); or alternatively it may be calculated from the well known equation for secretion rate, by means of the measured specific activity of the isolated metabolites (14). By either method the daily glandular secretion of 17-hydroxyprogesterone can be approximated as 3 mg. for the normal subject and between 240 and 280 mg. for the CAH patient. The lower estimate (derived from 17-hydroxypregnanolone) is probably the more reliable since the excretion of 17-hydroxypregnanolone was more nearly complete over the period examined. These estimates would be changed only slightly if the metabolites had been measured beyond the third day. This is evident from the small recovery of C^{14} and the low specific activity of the third day of collection. On the basis of these estimates, the CAH patient was secreting approximately 80 times the amount of 17-hydroxyprogesterone characteristic of a normal subject. These estimates are based upon the presumption that only 17-hydroxyprogesterone contributed significantly to the urinary metabolites pregnanetriol and 17-hydroxypregnanolone. It has been shown by Fotherby and Love (5) that $3\beta,17\alpha$-dihydroxy-Δ^5-pregnene-20-one is readily metabolized in a significant amount to pregnanetriol by human subjects. Thus the estimates of 17-hydroxyprogesterone production derived in this study would be in error by whatever $3\beta,17\alpha$-dihydroxy-Δ^5-pregnene-20-one contributed to the urinary pregnanetriol and 17-hydroxypregnanolone. Another metabolite of $3\beta,17\alpha$-dihydroxy-Δ^5-pregnene-20-one, the reduction product Δ^5-pregnene-$3\beta,17\alpha,20\alpha$-triol, was not found in the urine of this

subject—a fact that might be interpreted to indicate minor contribution from this potential precursor. Too much emphasis, however, should not be placed on the negative finding since, as indicated in a previous publication (15), separation of the nonketonic steroids from the urine of this subject is seriously complicated by the large amount of pregnanetriol. It is hoped to provide further information about this problem in future studies.

The transformation of 17-hydroxyprogesterone to the C_{19}-metabolites, androsterone and etiocholanolone, revealed a difference between the 2 subjects. As evident in Table 8, one man (Patient M) eliminated 1.7 per cent of the dose as these 2 metabolites, which together constituted 2.9 per cent of the neutral steroid fraction. The hyperplasia patient transformed 3.1 per cent of the administered 17-hydroxyprogesterone to androsterone and etiocholanolone, and these 2 metabolites together represented 6.3 per cent of the total neutral steroid fraction examined. In neither subject was complete recovery of these 2 metabolites achieved during the collection period, since in each subject androsterone and etiocholanolone comprised a considerable portion of the steroids in the urine of the third day. It could be presumed that excretion of these 2 compounds would have continued through the fourth, fifth and possibly subsequent days. Therefore, estimates of the contribution made by the endogenous secretion of 17-hydroxyprogesterone to these 2 major androgen metabolites will be minimal values, although probably not too far removed from the true amounts. With the normal subject this consideration is immaterial since, based upon pregnanetriol values, only an insignificant fraction of his daily total production of androsterone and etiocholanolone was formed from 17-hydroxyprogesterone. On the other hand, the CAH patient, who excreted a combined total of 16 mg. of androsterone and etiocholanolone, would have derived approximately 50 per cent of the endogenous production of these two 17-ketosteroids from 17-hydroxyprogesterone, as a consequence of the enormous secretion of precursor together with an apparently increased capacity to remove the 2 side-chain carbon atoms. A similar reservation must be made regarding this estimate, as was earlier noted for 17-hydroxyprogesterone production.

It is evident from the lower specific activity of both androsterone and etiocholanolone in comparison with the C_{21}-metabolites that a significant portion of each of these 17-ketosteroids is produced from other precursors than 17-hydroxyprogesterone. Among the more likely contributors are known hormones such as Δ^4-androstene-3,17-dione and dehydroisoandrosterone. There is a very significant difference in the metabolism of androsterone and etiocholanolone derived from 17-hydroxyprogesterone and the metabolism of an androgen such as testosterone. The 17-ketosteroid me-

tabolites of testosterone are promptly excreted in urine, the major quantity being eliminated within the first twelve hours (16). It is evident from the present study that the same metabolites derived from 17-hydroxyprogesterone appear very much more slowly and are found over a longer time. Whether this is a reflection of an enterohepatic circulation of other metabolic products of 17-hydroxyprogesterone, or of some retention in tissues, or of a metabolic peculiarity of the CAH patient cannot be decided from the available information. Studies designed to clarify this problem are in progress. It should be noted, however, that the pattern of urinary excretion of the metabolites of 17-hydroxyprogesterone, as well as the rather large amount of radioactivity in the feces, are characteristic of steroids that undergo an enterohepatic circulation, for example, estrogens (17) and progesterone (18).

These considerations raise a question about the hormonal control of virilization of the CAH patient. Although the virilization had been established *in utero* (19) and in the strictest sense the present results apply to the adult individual, it seems reasonable that similar factors were operative during fetal development and early childhood. Even with due allowance for the androgen metabolites derived from 17-hydroxyprogesterone, it is clear that the endogenous production of these same compounds from other precursors was at a high level for a normal male and well above that characteristic of a normal female. Apparently an already elevated level of secretion of C_{19} adrenal androgen was augmented by the metabolic transformation of a portion of the excessive 17-hydroxyprogesterone secretion. The adrenal androgen secretion would be more than sufficient to maintain the pattern of virilism already established. The additional influence exerted by the androgen metabolites of 17-hydroxyprogesterone is speculative but in view of the demonstrated biologic activity of steroid metabolites, as demonstrated by other work in these laboratories, this contribution clearly should be considered (20, 21). The slight evidence for a difference in metabolism of 17-hydroxyprogesterone in the CAH patient may well be pertinent. It is plausible that this is one of the several influences resulting from the very large production of unusual steroids characteristic of this disorder. One cannot, however, disregard the relative deficiency in hydrocortisone production as a factor that may influence the metabolic transformation of other hormones. Although these should be simply considered as suggestions for further work, it is evident that there are many intriguing biochemical and physiologic problems which remain to be elucidated in this abnormality of steroid production.

Addendum

Since the submission of this manuscript R. V. Brooks (*J. Endocrinol.* **21**: 277, 1960) has published studies of the metabolism of large doses of 17-hydroxyprogesterone ad-

ministered sublingually to a patient with "post-pubertal adrenal virilism having a pattern of steroid excretion characteristic of congenital adrenal hyperplasia" and to a normal subject. His results with unlabeled steroid were in agreement with those reported in the present study for tracer doses.

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EFFECTS OF PREGNANCY ON IODINE METABOLISM IN THE PRIMATE*†

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ABSTRACT

In contrast to the human, the rhesus macaque exhibits rapid thyroidal accumulation of I^{131} which is not augmented by pregnancy. The effects of pregnancy on several parameters of iodine metabolism in the macaque are similar to the effects observed during estrogen therapy in nonpregnant humans. These are: an increase in thyroxine binding by the thyroxine-binding globulin, slowing of the fractional rate of thyroxine turnover, and an increase in the concentration of circulating hormone without an appreciable change in the volume of distribution of thyroxine. Accordingly, the net daily hormonal turnover is no different during pregnancy than in the nonpregnant animal. A similar phenomenon was noted in 2 of 3 women similarly studied during and after therapeutic abortions. In the first woman there was an appreciable slowing of the fractional rate of thyroxine turnover during pregnancy, with a return to normal values after abortion. In the 2 other women, who suffered significant intercurrent illnesses, the rates were characteristic of normal nonpregnant subjects. In 1 of these 2 women, the rate was further accelerated during the postpartum follow-up period. Consequently, the net quantity of hormone turned over daily was the same during pregnancy as it was post partum. However, in the other woman the net hormonal turnover was markedly reduced at the time of postpartum follow-up examination. At the time of abortion, the concentration of organic I^{131} in fetal serum was approximately one-fifth that in maternal serum.

THE findings in pregnant women of thyromegaly (1), increased thyroidal avidity for I^{131} (2–4), and an increased concentration of protein-bound iodine (PBI) in the serum (5) indicate that there is increased pro-

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duction and utilization of thyroxine during pregnancy. Recently it has been found that large doses of estrogen given to nonpregnant subjects induce parallel increases in the concentration of serum PBI and in the thyroxine-binding capacity of the thyroxine-binding globulin of serum (TBG). These changes are quantitatively similar to the increases characteristic of pregnancy (6, 7). Yet, in contrast to the effects of pregnancy, diethylstilbestrol has no effect on the thyroidal uptake of I^{131} or on the calculated daily incorporation of I^{127} (8). Coupled with the observation that estrogenic therapy induces an increase in the concentration of serum PBI in athyrotic patients receiving replacement therapy with desiccated thyroid, these findings suggested that most if not all of the increased concentration of circulating hormone must result from slowing of the fractional rate of thyroxine turnover rather than from increased thyroidal elaboration of hormone (9). This postulate was directly proved by studies of the disappearance from plasma of I^{131}-labeled thyroxine (10, 11). The question next was raised: does a similar phenomenon occur during human pregnancy or do other factors exist which lead to the utilization and degradation of increased quantities of thyroxine?

To answer this question, studies of thyroxine metabolism were performed in the pregnant subhuman primate. These revealed that the fractional rate of peripheral turnover of the hormone is retarded during pregnancy. Further, the magnitude of the effect is such that when the increase in the concentration of circulating hormone is considered, the net daily quantity of thyroxine utilized or degraded does not differ from that in the nonpregnant state. A similar phenomenon was observed in 2 of 3 women studied before and after therapeutic abortion.

MATERIALS AND METHODS

Thyroxine metabolism was investigated, by techniques previously described (11, 12), in the pregnant woman and in the rhesus macaque. Among the macaques, 7 nonpregnant animals were compared with 8 pregnant ones; 3 of these animals underwent paired studies before and during, or during and after pregnancy.

To minimize thyroidal recycling of I^{131} peripherally liberated from labeled thyroxine, a dose of 3 to 5 drops of a saturated solution of potassium iodide was given daily to the animals during study, and 30 mg. of methimazole every six hours to pregnant women. Samples of blood were drawn twice daily from the animals for five to seven days, and daily from the women for seven to ten days. Concentrations of butanol-extractable iodine in the sera were measured at the beginning and the end of each period of study.[1] Serum thyroxine-binding by TBG and by the thyroxine-binding pre-albumin (TBPA) was assessed by zonal electrophoretic techniques described elsewhere (13).

Thyroidal accumulations of I^{131} and I^{127} were compared in 3 pregnant and 3 nonpregnant animals by the following techniques. An animal was placed in a plastic collar

[1] Kindly measured by Bio-Science Laboratories, 12330 Santa Monica Boulevard, Los Angeles 25, California.

which fixed its head and neck in the center of the metabolism cage. During measurement of I^{131} accumulation, the animal's hands were restrained by canvas binders to the sides of the cage. After the intravenous injection of a tracer dose of I^{131}, one or two consecutive 24-hour urines were collected. Counts of radioactivity over the thyroid were made at intervals ranging from five minutes to twenty-four hours by positioning a scintillation detector directly against the front edge of the plastic collar, a distance of 15 cm. from the thyroid region. Vascular background was estimated by making counts at the same distance from the restrained right hand. The quantities of I^{131} and I^{127} excreted in the urine were measured and the thyroidal incorporation of I^{127} was calculated by methods described elsewhere (14).

In order to ascertain the nature of the circulating organic iodine in the pregnant monkey, sera were obtained forty-eight and seventy-two hours after the administration of 25 microcuries of I^{131}. The serum was subjected to extraction with acidic butanol and the extract was evaporated to dryness at low temperature. Each residue was taken up in 1.0 ml. of ammoniacal methanol (2*N* NH_4OH:methanol, 1:7 v/v), and 0.1 ml. of the solution was chromatographed with carrier organic iodine and iodide[2] on strips of Whatman #3 filter paper in two solvent systems—butanol:acetic acid:water (78:10:12 v/v), and butanol:dioxane:ammonia (80:20 v/v saturated with 2*N* NH_4OH). Compounds were localized by dividing the strips into 1.0-cm. segments and counting radioactivity in the segments with a well-counter. Duplicate strips were stained with diazotized sulphanilic acid (prepared by mixing in the cold, before use, 1 part of 4.5 per cent sodium nitrite and 5 parts of 0.8 per cent sulfanilic acid in 9 per cent concentrated hydrochloric acid, v/v) and sodium carbonate (10 per cent) after localization of iodide with palladium chloride (0.1 per cent, acidified with HCl). The results are shown in Figure 1.

In the human studies, during each therapeutic abortion, blood was obtained from the fetus and the level of protein-bound I^{131} (PBI^{131}) was measured after precipitation of proteins from 3.0-ml. aliquots of serum with 10 per cent trichloracetic acid (TCA) followed by multiple washings of the precipitate with 5 per cent TCA. The concentration of PBI^{131} per liter of fetal serum was expressed as a percentage of the dose of I^{131} administered to the mother.

Sera obtained from the pregnant women during each of the periods of study were diluted fourfold by the addition of Krebs-Ringer-phosphate buffer, pH 7.4. Each sample of buffer-diluted serum was divided into two aliquots of 10 ml. each. Tracer thyroxine was added to one aliquot. This sample was placed in a dialysis bag and dialyzed against the unlabeled diluted serum. The rate of transfer of radioactivity to the dialysate was assessed by frequent sampling from the dialysate (15).

RESULTS

Iodine metabolism in the rhesus macaque

Thyroidal I^{131} and I^{127} accumulation. Data from individual animals, including calculations of thyroidal incorporation of I^{127}, are summarized in Table 1. In contrast to the human thyroid gland, the initial accumulation of I^{131} by the macaque thyroid gland was extremely rapid, maximal accu-

[2] The carrier solution was prepared as follows: thyroxine, 2 mg.; triidothyronine, 2 mg.; monoiodotyrosine, 1 mg.; diiodotyrosine, 1 mg.; potassium iodide, 0.5 mg.; propylthiouracil, 0.8 mg.; dissolved in 1.0 ml. of 2*N* NH_4OH:methanol, 1:7 v/v. The amount of this solution applied to each chromatogram was 50 μl.

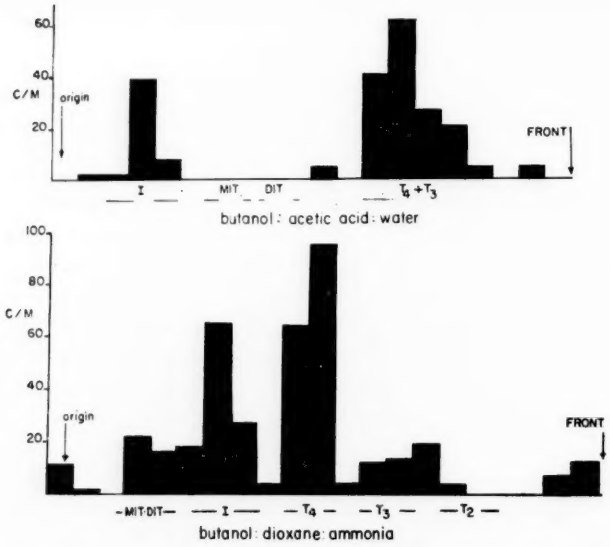

FIG. 1. Values for serum I^{131} forty-eight hours after intravenous administration of sodium-I^{131} to the pregnant macaque.

mulation occurring within thirty minutes after administration of the tracer. In all animals the uptake of I^{131} during the first twelve hours ranged from 13 to 21 per cent of the administered dose; values at twenty-four hours ranged from 10 to 24 per cent. The 24-hour urinary excretion of I^{127} ranged from 27 to 61 μg. In nonpregnant animals the 24-hour thyroidal accumulation of I^{127} ranged from 11.4 to 19.9 μg., and in pregnant animals from 9.2 to 16.2 μg. It is unfortunate that clearance studies could not be performed without anesthetizing or sedating the animals, since it became apparent that thyroidal iodide was released during the periods of urine collection. Thus, the calculations of thyroidal incorporation of I^{127} lose validity. Furthermore, the required physical restraint may have affected iodide metabolism in a manner which obliterated possible underlying differences between nonpregnant and pregnant animals. Within these limitations it was concluded, however, that a difference in the thyroidal uptake of iodide between nonpregnant and pregnant animals was not established. As in the human, the principal circulating organic compound is thyroxine.

Kinetics of thyroxine metabolism. Data obtained from the study of thyroxine metabolism in the macaques are presented in Table 2. For purposes of comparison, replicate studies performed at different times in the same animal are included. In the present communication, mean values derived from these studies were employed for statistical analysis.

TABLE 1. THYROIDAL I^{131} AND ESTIMATED I^{127} ACCUMULATION IN NONPREGNANT AND PREGNANT MACAQUES

Animal	Stage of pregnancy	Thyroidal I^{131} (% dose)		Urinary I^{131} (% dose/24 hrs.)	Urinary I^{127} (μg./24 hrs.)	Estimated thyroidal I^{127} (μg./24 hrs.)
		1 hr.	24 hrs.			
Nonpregnant						
Nancy	—	17	18	55.2	61*	19.9
Sarah	—	20	24	56.0 20.4	37 45	17.6
Mrs. Pettibone	—	16†	10	59.1 20.3	43 40	11.4
Evelyn‡	—	19	—	—	—	
Pregnant						
Mother Tucker	Early	19	19	73.9 10.0	45 43	11.3
Fanny	Term	13	24	65.1	44	16.2
Charlotte	Early	21	21	61.7	27	9.2

* When consecutive daily urinary I^{127} excretions were determined, mean values were employed in calculations.
† One-hour uptake employed in calculations.
‡ Animal strangled in collar.

Since paired studies could be made in only 3 animals, unpaired data were accumulated for statistical comparison (Table 3). It is noteworthy, in the 3 paired studies, that inconsistencies appear in 2 cases. Thus, in macaque Molly, ten days after abortion the level of serum butanol-extractable iodine (BEI) was reduced, although the fractional rate of hormonal turnover (k) was retarded when compared with the study performed ten weeks after abortion. Similarly, in Mrs. Pettibone, the mean values for k and BEI during pregnancy did not differ appreciably from those found six weeks post partum, although thyroxine binding had decreased. Despite inclusion of these data in the statistical comparison of the nonpregnant and pregnant goups, the differences between the groups are striking.

The thyroxine distribution space (TDS) was proportional to body weight; it represented 10.7 ± 1.2 per cent of body weight in nonpregnant, and 9.2 ± 1.0 per cent in pregnant animals. This difference was not statistically significant. The fractional rate of peripheral turnover of hormone (k) was markedly reduced during pregnancy. The volume turnover of hormone or the clearance rate (C) consequently was also significantly reduced. When expressed in terms of body weight, this difference remained significant. Concomitant with slowing of the peripheral turnover of hormone there was a reciprocal increase in the serum concentration of BEI. As a consequence of these reciprocal changes in the clearance rate and the concentra-

TABLE 2. SEVERAL PARAMETERS OF IODINE METABOLISM IN THE RHESUS MACAQUE

Animal	Duration of pregnancy or period of study* (mos.)	Wt. (Kg.)	TDS†		k (%/day)	C (ml./day)	C/Kg. (ml./Kg./day)	BEI (μg./100 ml.)	D (μg. I/day)	D/Kg. (μg. I/Kg./day)	Serum thyroxine-binding (saturation capacity, μg. T_4 %)	
			(ml.)	(% wt.)							TBG	TBPA
Nonpreguant												
1. Tony		5.0	470	9.4	36.6	173	34	2.9	5.0	0.99	29	115
	1	5.0	520	10.4	31.2	163	32	2.7	4.3	0.86	23	132
	3	5.0	590	11.8	36.6	216	43	3.4	7.5	1.50	24	87
	11	5.3	650	12.2	33.0	214	40	3.1	6.6	1.24	26	91
Mean		*5.1*	*558*	*10.9*	*34.4*	*192*	*37*	*3.0*	*5.8*	*1.15*	*26*	*106*
2. Margie		5.0	480	9.6	46.0	210	44	2.9	6.5	1.30	27	40
	1	5.0	620	12.4	34.7	217	43	1.8	4.0	0.79	29	39
	9	5.5	530	9.8	36.5	193	35	2.3	4.5	0.81	30	50
Mean		*5.2*	*543*	*10.6*	*39.1*	*207*	*41*	*2.3*	*5.0*	*0.97*	*29*	*43*
3. Nancy		5.7	470	8.3	27.7	130	23	2.9	3.8	0.67	32	125
	1	5.8	530	9.3	30.5	163	28	3.5	5.6	0.98	23	120
Mean		*5.7*	*500*	*8.8*	*29.1*	*146*	*25*	*3.2*	*4.7*	*0.83*	*28*	*122*
4. Molly	10 days after abortion	3.4	360	10.5	26.6	96	26	2.3	2.2	0.64	34	82
	10 weeks after abortion	3.4	380	11.2	38.5	146	43	3.3	4.8	1.40	25	46
Mean		*3.4*	*370*	*10.9*	*32.5*	*121*	*35*	*2.8*	*3.5*	*1.02*	*29*	*64*
5. Mrs. Pettibone	6 weeks post partum	4.4	560	12.7	23.9	134	30	3.3	4.4	0.99	39	91
6. Melva		4.1	430	10.4	38.5	166	40	2.6	4.3	1.04	24	98
7. Marilyn		4.1	420	10.4	38.5	162	40	2.4	3.9	0.95	27	76
Pregnant												
1. Molly	2	3.6	320	8.9	23.6	76	21	4.5	3.4	0.95	80	82
2. Mrs. Pettibone	3½	4.1	340	8.3	24.7	84	21	4.0	3.4	0.83	60	120
	4½	4.5	590	13.5	19.8	117	27	3.1	3.8	0.84	52	95
Mean		*4.3*	*465*	*10.9*	*22.2*	*100*	*24*	*3.5*	*3.6*	*0.84*	*56*	*108*
3. Arlene	3½	5.0	330	6.6	24.7	82	16	6.2	5.0	0.99	43	140
	4½	5.0	440	8.8	23.9	110	21	4.3	4.5	0.90	55	125
Mean		*5.0*	*385*	*7.7*	*24.3*	*96*	*18*	*5.2*	*4.8*	*0.95*	*49*	*132*
4. Margaret Rose	4½	4.1	380	9.3	24.7	94	23	4.2	4.0	0.97	58	96
5. Nancy	1½	5.5	560	10.2	27.7	156	28	4.5	7.2	1.30	65	110
6. Shirley	3½	5.2	440	8.5	25.7	113	22	4.0	4.6	0.88	55	130
7. Fanny	3½	5.2	450	8.7	23.4	105	20	3.5	3.6	0.70	37	150
8. Katy	5½	4.8	440	9.1	31.5	139	29	4.5	6.3	1.30	59	82

* Average duration of macaque pregnancy is approximately 5½ mos. (164 days).
† For definition of abbreviations, see text and Appendix.

TABLE 3. STATISTICAL COMPARISONS OF SEVERAL PARAMETERS OF IODINE METABOLISM IN NONPREGNANT AND PREGNANT MACQUES

	TDS*		k	C	C/Kg.
	(ml.)	(% wt.)	(%/day)	(ml./day)	(ml./Kg./day
Nonpregnant	483 ± 77†	10.7 ± 1.2	33.0 ± 5.3	161 ± 31	35 ± 6
Pregnant	430 ± 71	9.2 ± 1.0	25.4 ± 3.0	110 ± 26	23 ± 4
P	>0.1	>0.1	<0.01	<0.01	<0.01

	BEI (μg./100 ml.	D (μg. I/day)	D/Kg. (μg. I/Kg./day)	TBG (μg. T_4 %)	TBPA (μg. T_4 %)
Nonpregnant	2.8 ± 0.4	4.5 ± 0.8	0.99 ± 0.10	29 ± 5	86 ± 29
Pregnant	4.2 ± 0.6	4.7 ± 1.4	0.99 ± 0.08	57 ± 12	111 ± 25
P	<0.01	>0.1	>0.1	<0.01	>0.1

* For definition of abbreviations see text and Appendix.
† Mean and standard deviation.

tion of circulating hormone, the daily degradation of hormone (D) was unchanged, whether expressed as total degradation or in terms of body weight. In the latter terms, the value of D was 0.99 ± 0.10 μg. of iodine per Kg. per day in nonpregnant animals,[3] and 0.99 ± 0.08 μg. of iodine per Kg. per day in pregnant animals. As in human pregnancy, there was a comparable increase in the saturation capacity of circulating TBG for thyroxine. The thyroxine-binding capacity of TBG and of TBPA was of the same magnitude as that found in the human. Similarly, there was no net change in the saturation capacity of TBPA for thyroxine.

TABLE 4. SEVERAL PARAMETERS OF IODINE METABOLISM DURING AND AFTER HUMAN PREGNANCY

Patient	Diagnosis	Status	TDS* (L.)	k (%/day)	C (ml./day)	BEI (μg./100 ml.)	D (μg. I day)	TBG (μg. T_4 %)	TBPA (μg. T_4 %)	Serum fetal/maternal organic I^{131} ratio
D.M.	Huntington's chorea	Pregnant (28 wks.);	9.2	8.2	754	6.9	52	41	78	0.24†
		post-abortal (1 wk.)	8.2	11.4	935	5.8	54	23	86	—
V.M.	Cervical carcinoma	Pregnant (29 wks.);	8.5	11.4	969	7.5	73	43	38	0.19
		post-abortal (3 wks.)	8.3	15.7	1,303	5.7	74	22	47	—
T.P.	Hyperemesis gravidarum	Pregnant (25 wks.);	8.6	11.1	955	7.7	74	35	32	—
		post-abortal (14 wks.)	7.8	12.6	983	4.4	43	20	71	—

* For definition of abbreviations see text and Appendix.
† Determined at time of abortion.

[3] Comparable values in the eumetabolic human subject range from approximately 0.6 to 1.2 μg. of iodine per Kg. per day.

Thyroxine metabolism in the pregnant woman

Data derived from the studies on 3 pregnant women are summarized in Table 4. In Patient D.M., the fractional rate of thyroxine turnover (k) was markedly reduced but had returned to a normal value one week after abortion. The volume of distribution of thyroxine (TDS) decreased slightly. Concomitant with the acceleration in the rate of thyroxine turnover there was a corresponding decrease in the concentration of serum BEI. As in the pregnant macaque, the combination of these reciprocal changes in the rate of turnover and concentration of circulating hormone resulted in no net difference between the turnover of hormone during pregnancy and post partum. As noted earlier (6, 7), there was marked augmentation in the thyroxine-binding capacity of TBG during pregnancy. On the other hand, there was no appreciable difference between the thyroxine-binding capacity of TBPA during and following pregnancy. Values also were comparable to those obtained in other nonpregnant subjects.

The data on the other 2 women (V.M. and T.P.) differed from those on Patient D.M. The fractional rate of thyroxine turnover (k) in Patients V.M. and T.P. during pregnancy was within the range found in *nonpregnant* eumetabolic subjects. However, in these 2 patients during the postpartum follow-up period the fractional rate of thyroxine turnover was accelerated beyond that found during their pregnancies. As in Patient D.M., the volume of distribution of hormone (TDS) in these women was slightly reduced, and in Patient V.M. the concentration of serum BEI was decreased by such an increment that the net daily degradation of thyroxine did not differ in the postpartum period from that during pregnancy. In Patient T.P., however, there was a marked decrease in the concentration of serum BEI with only a small acceleration in the fractional rate of thyroxine turnover. Consequently, the net daily turnover of hormonal iodine was markedly reduced in the postpartum follow-up period.

Of interest in Patients V.M. and T.P. was the marked reduction in the thyroxine-binding capacity of serum TBPA during pregnancy. This finding contrasts with the normal values observed in Patient D.M., who underwent therapeutic abortion because of mental deterioration due to Huntington's chorea; her pregnancy otherwise was uncomplicated. It is noteworthy that the other 2 women suffered significant intercurrent illnesses. Thus, Patient V.M. underwent therapeutic abortion preparatory to treatment for carcinoma of the cervix, and Patient T.P. underwent abortion for severe hyperemesis. Confirmatory of the decreased thyroxine-binding capacity detected by zonal electrophoresis was the observation of accelerated transfer of tracer thyroxine across a cellophane membrane between sera obtained from Patient V.M. before and after pregnancy (Fig. 2).

As noted by others (16, 17), the concentration of tracer per liter of fetal serum was approximately one-fifth of the concentration per liter of maternal serum.

DISCUSSION

The few data from the studies of the pregnant women do not firmly establish that turnover of hormonal iodine is unaffected by pregnancy. It is conceivable that there may be periods during pregnancy other than those studied in which there is increased peripheral utilization and degradation of hormone, or that the times of post-abortal study did not serve as suitable control periods. When combined with the data on the rhesus

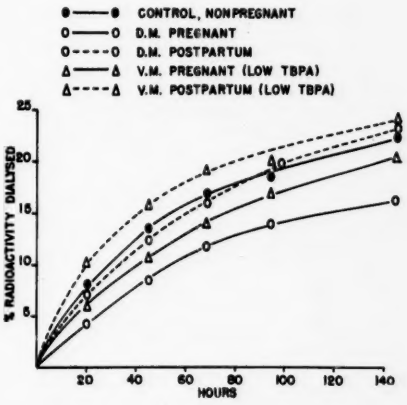

FIG. 2. The transfer of thyroxine-I^{131} radioactivity across a cellophane membrane between sera (diluted with buffer) obtained from women during and after pregnancy. Note a) the somewhat more rapid transfer between the sera of Patient V.M. during pregnancy than between the sera from the pregnant control, D.M.; and b) the more rapid transfer between the postpartum sera of Patient V.M. than between the postpartum sera of Patient D.M.

macaque, the present observations are compatible with the hypothesis that the increase in concentration of circulating thyroid hormone during primate pregnancy results primarily from a decrease in the fractional rate of thyroxine turnover. During the periods of study the daily turnover of hormonal iodine appeared not to differ from that in the nonpregnant state. The phenomenon is thus analogous to that observed in nonpregnant subjects given large doses of natural or synthetic estrogens (9). That the proximate cause of these effects may be an estrogen-induced increase in the thyroxine-binding capacity of TBG is suggested by the finding that in a case of congenital absence of TBG, the administration of large doses of estrogen failed to produce either detectable levels of TBG or retardation of the fractional rate of thyroxine turnover (Ingbar, S. H., unpublished observations).

In 2 of the 3 pregnant women the rates of thyroxine turnover were similar to those reported for eumetabolic nonpregnant persons. These normal rates

occurred despite the fact that the characteristic increase in the thyroxine-binding capacity of TBG was found. The two observable differences between these women and the other pregnant women, or the animals studied, were: 1) the presence of serious intercurrent illness, and 2) the finding of subnormal saturation capacities of TBPA. The significance of these factors was explored further by dialysis techniques wherein the rate of transfer of tracer thyroxine from serum to serum across a cellophane membrane was observed (Fig. 2). This measure of serum thyroxine binding, performed at physiologic pH, confirmed the observation of diminished protein binding of thyroxine made by zonal electrophoresis. Thus, the rate of transfer of labeled thyroxine across the membrane was more rapid than that between aliquots of serum from a normally pregnant woman. Recently it has been observed that thyroxine binding by TBPA is reduced in a variety of acute and chronic illnesses and that, in most instances in which studies have been performed, the fractional rate of turnover of hormone is accelerated (18). It is noteworthy that the duration of the intercurrent illness differed in Patients V.M. and T.P. Thus, untreated cervical carcinoma was present during both studies of Patient V.M., whereas hyperemesis existed only during the pregnancy of Patient T.P., and at the time of follow-up examination she was clinically well. Similarly, thyroxine binding by TBPA was reduced during both studies of Patient V.M., but was appreciably reduced only during the pregnancy of Patient T.P. In Patient T.P., the finding of a greater net turnover of hormonal iodine during pregnancy than at the time of follow-up examination may reflect, not an effect of pregnancy *per se* but rather a consequence of her intercurrent illness. Since the significance of altered thyroxine binding by TBPA as demonstrated electrophoretically has not been directly established (19), a relationship between the low capacities of TBPA and the relatively accelerated turnover of hormone cannot be assured. In these women the possibility exists, however, that the acceleration in the fractional rate of thyroxine turnover resulted from decreased thyroxine binding by this moiety.

When considering factors which may affect the fractional rate of peripheral thyroxine turnover, it is germane to emphasize the importance of factors other than alterations in serum thyroxine binding. Some of these are suggested from the present study. Although the daily turnover of hormonal iodine is unchanged during pregnancy, the fractional rate of thyroxine turnover is inversely proportional to thyroxine binding by TBG. When these parameters are compared in the monkey and in man, it is noted that whereas serum thyroxine binding and the daily hormonal turnover per kilogram of body weight are similar, the fractional rate of thyroxine turnover is several times more rapid in the monkey. Thus, either peripheral tissue degradation of hormone is more rapid in the macaque or

there exists a difference in serum thyroxine binding between the two primates which is not demonstrable by the techniques employed. Alternatively, the difference might result from a more rapid turnover of circulating thyroxine-binding proteins.

Although the present studies partly elucidate the causes of altered iodine metabolism during pregnancy, several conflicting observations remain to be explained. That the pregnant macaque may not be a complete model for the effects of human pregnancy is suggested by the failure to find in the pregnant animal, at least grossly increased thyroidal avidity for I^{131}. Assuming within the limitations of the data that this be true, the pregnant macaque is an analogue of the estrogen-treated nonpregnant human, but not of the pregnant woman.

In the estrogen-treated human, mechanisms may exist for temporarily increasing the thyroidal contribution to the circulating thyroxine pool during the period when serum thyroxine binding is increasing. Thus, it was found that during suppression of the pituitary-thyroid system with triiodothyronine, the estrogen-induced increase in thyroxine binding by TBG is associated with a small but significant acceleration in the rate of loss of I^{131} from the thyroid (20). It was postulated that if proteolysis of thyroglobulin were not rate-limiting, increased thyroxine binding by the circulating TBG perfusing the gland would favor the exchange of thyroxine (surface-bound to thyroglobulin) across the follicular cell and vascular endothelium into the circulation. This hypothetical event, by reducing thyroidal stores of hormone, should be followed by a compensatory increase in thyroidal avidity for iodide. Such a phenomenon could contribute during early pregnancy to the attainment of the new level of circulating hormone. However, in the absence of increased peripheral demands for hormone, once the new equilibrium was achieved, the need for continuing the increased thyroidal contribution would no longer exist.

Although measurements of thyroidal I^{131} uptake were not possible during the present studies of human pregnancy, thyroxine metabolism was assessed during a period when (as shown in earlier work) augmented thyroidal avidity for I^{131} should have been present (2, 3, 4, 14). What, then, is the significance of the increased functioning of the thyroid gland in normally pregnant women if it occurs at a time when no increase in the daily turnover of hormonal iodine can be demonstrated? The findings in pregnant women are most consistent with the hypothesis earlier proposed (8)—that a part of the augmented functioning of the gland may result from a relative deficiency of circulating iodide imposed by losses of the anion to the conceptus and by the profound hemodynamic alterations which occur during pregnancy. Other possible factors that may contribute to the phenomenon remain enigmatic.

Acknowledgments

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APPENDIX

In the foregoing presentation, the following terms and calculations were employed: TDS (thyroxine distribution space), the virtual volume of distribution of exchangeable thyroxine considered to be present in the same concentration in which it exists in the serum. BEI (butanol-extractable iodine), the concentration of hormonal iodine in the serum and, by definition, the mean concentration of hormonal iodine in the TDS. The k (fractional rate of turnover of thyroxine)—the percentage of the hormonal iodine content of the TDS removed daily, here equal to 100×0.693/the time required for removal of half of the hormonal iodine content of the TDS. C (thyroxine clearance), the volume of serum which contains an amount of thyroxine equal to that removed daily from the TDS ($C = TDS \times k$). D, hormonal iodine removed from the TDS daily by the action of all mechanisms for its disposal ($D = C \times BEI$). In animal experiments, because of variations in body weight, TDS, C and D were expressed in terms of body weight.

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THYROID ANTIBODIES DURING PREGNANCY AND IN THE NEWBORN*

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ABSTRACT

Twenty-two women, found to have circulating antithyroid antibody (AAB) either before or during pregnancy, were observed during 23 pregnancies. The maternal AAB titers began to fall as early as the first trimester, tended to strike a plateau at a low level in the third trimester, and rose again during the first few months after the termination of pregnancy. These antibodies were demonstrated in the cord blood of 8 newborn infants of 9 mothers who had demonstrable circulating AAB at the time of delivery, but could not be demonstrated in the cord blood of 7 newborn infants of mothers whose AAB titers had fallen to zero by the time of delivery. AAB disappeared from the blood of all infants during the first 3 months of life. No clinical evidence of hypothyroidism was found in the living offspring at birth or at the time of follow-up examination for as long as 2 years after birth. The levels of serum protein-bound iodine at birth in 6 newborn infants with proved transplacental transfer of AAB, and later in 4 of these subjects and 2 other offspring, were within the normal range for the ages concerned. It is concluded that the presence of AAB in the maternal and fetal circulations is not a cause of cretinism.

THE hypothesis that transplacental transfer of maternal thyroid auto-antibodies (AAB) may produce fetal hypothyroidism was advanced by Beierwaltes *et al.* (1, 2), and subsequently tested extensively by Blizzard and his associates (3). Both groups of investigators found a comparable incidence of thyroid AAB in mothers of cretins. Blizzard reported an incidence significantly higher than in the general population and higher than in what he considered to be a "control" pregnant population. Blizzard's data also support the thesis that transplacental transfer of thyroid AAB does occur and that the antibodies rapidly disappear from the infant's blood.

In general, these studies were dependent on data obtained at the time of

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delivery or later. Since the hypothesis being tested is that damage to the fetal thyroid may occur *in utero*, perhaps early in embryonic development, such studies are somewhat retrospective. This report presents observations on the titers of circulating thyroid AAB in women before, during and after pregnancy, with information on the transplacental transfer of AAB.

METHODS

Patients

Pregnant women with demonstrable circulating AAB were located by two means: 1) Screening of the women of child-bearing age afflicted with thyroid disease, who were seen in the Clinical Radioisotope Unit of the University Hospital; the selected group were either pregnant when first seen and found to have thyroid AAB or they subsequently became pregnant. 2) Routine testing, at Women's Hospital (University of Michigan Medical Center), of the sera of 400 obstetrical registrants for a first prenatal examination.

An effort was made to obtain blood from each mother at the time of: (*a*) every prepartum examination, (*b*) delivery, and (*c*) at least one postpartum visit. Blood was tested at the time of delivery in all mothers exhibiting AAB late in pregnancy, and in most mothers in whom titers that were demonstrable at the onset of pregnancy dropped to undetectable levels by the time of delivery.

Serum protein-bound iodine (PBI) determinations were made on cord blood at delivery in 6 of 8 cases in which AAB titers were demonstrable in the cord blood. Five infants were followed further with serum PBI and antibody determinations, made by the pediatrician at the time of their first postnatal check-ups at ages 3 to 12 weeks. Information regarding growth and development to 6 weeks of age or beyond was available in all but one of the living infants.

Sera were also obtained from 100 women, aged 18 to 35, who registered in the outpatient clinic of the University Hospital. This group served as a "control" for approximation of the incidence of demonstrable circulating AAB in nonpregnant women of the same age as those in the study.

Serology

Blood was centrifuged immediately after clotting and sera were frozen at $-10°$ C. In a few instances the freezing was delayed because the sera were sent to us by airmail.

Thyroid antigen was prepared by the method of Witebsky *et al.* (4), from human exophthalmic goiter obtained at the time of surgical thyroidectomy. Witebsky's modification of Boyden's tanned red blood cell hemagglutination technique (4) was used initially to demonstrate the presence or absence of circulating AAB. Titers are expressed as reciprocals of the greatest dilution of serum clearly producing agglutination.

During the course of the study (September 1957 to December 1960), three antigens were used for testing. Because these antigens differed in potency, all of the available remaining sera tested with the first two antigens were retested at the conclusion of the study with the third antigen in current use. In some instances, antibody determinations could not be repeated because the sera had been consumed in performing the serum PBI and inorganic iodine determinations at the time of delivery. In order to complete Table 1 and Figure 1, seven such titers (marked with an asterisk) were corrected for variations in comparative antigenic potency of the three antigens used during the three-year period of this study.

TABLE 1. PERTINENT DATA ON 22 MOTHERS AND 18 OF THEIR INFANTS

(These women, found to have circulating antithyroid antibodies either before or during pregnancy, were observed during 23 pregnancies.)

	Diagnosis & treatment	AAB titers before pregnancy	AAB titers—weeks before delivery																			
			38	34	32	30	28	26	24	22	20	18	16									
QUANTITATIVE																						
D.B.	1) Hashimoto thyroiditis; desicc. thyroid, 180 mg./day						640				320											
	2) Hashimoto thyroiditis; desicc. thyroid, 180 mg./day	80 at 6 mos.				80			20			5										
H.W.	Hashimoto thyroiditis; desicc. thyroid, 180 mg./day	320 at 1 mos.	80				80		40		10											
J.G.	Hashimoto thyroiditis; desicc. thyroid, 180 mg./day	5120 at 15 & 16 mos.			1280		1280	1280		1280		1280										
C.K.	Normal					640				160			160									
L.S.	Normal								320			160		80								
C.M.	Normal											40										
G.H.	Normal									320		160										
M.J.	Normal				40		0															
D.C.	See Text										160											
L.R.	Normal											80										
P.H.	Hashimoto thyroiditis; desicc. thyroid, 180 mg./day	640 at 2 yrs.							320		320			80								
U.H.	Normal					20																
R.D.	Normal								10													
I.C.	Normal										5											
L.B.	Normal mother of cretin by previous pregnancy	10 at 6 mos.									0											
E.A.	Normal				1280			640	Aborted		320											
NOT QUANTITATIVE																						
M.K.	Hashimoto thyroiditis; desicc. thyroid, 180 mg./day	24 at 7 mos.				0		0		0			0									
J.R.	Obese, normal; ? taking desicc. thyroid						28					384										
U.G.	Hashimoto thyroiditis; desicc. thyroid, 180 mg./day						48	0			0											
J.M.	Normal							27				0										
B.L.	Normal									3												
P.T.	Normal								9													

TABLE 1—(*Continued*)

AAB titers—weeks before delivery									Serum PBI at term (μg./100 ml.)	AAB titers—months after delivery										Duration of infant follow-up
14	12	10	8	6	4	2	Term			1	2	3	4	5	6	7	8	9	10	
QUANTITATIVE																				
	40			0			Infant	80	80 160			640 0	1280 (PBI, 6.8)					40		2 yrs.
		0			0	10	Infant		10 5											6 wks.
0			0			0 Infant			0 0			40				5				7 mos.
	320			320		40 Infant			40 80	7.5		40								6 wks.
	160		80			Infant			160* 80*	9.1 8.9	320 0	320 (PBI,7.2)								2 mos.
			80		80	80 Infant			80 20*	10.0 6.9		160 0 (PBI, 5.5)								3 mos.
				40		Infant			40 5	7.1 8.1										1 yr.
			40			Infant			80* 40*	9.7 8.5		320 0 (PBI, 6.7)								2 mos.
		0							0											6 wks.
	160		160			Hydramnios Infant			120* 40*	9.3 9.7	0 (PBI,8.0)		640	640	1280	2560 5120		320	2560	10 mos.
						Infant			0 0											1 yr.
																				6 mos.
		0																		6 wks.
						Infant died from erythroblastosis fetalis		0												—
								0												8 mos.
						0 Infant			0 0		0 (PBI, 7.7)									2 yrs.
																				—
NOT QUANTITATIVE																				
	0		0			Infant			0 0		0									6 wks.
		0				Infant			0 0											1 yr.
		0		6					0			0								3 mos.
	24			27		27 Infant			20 0			0								6 wks.
					0	Infant			0 0											5 days
					0	Infant			0 0			0								6 wks.

* Titers corrected for variations in comparative antigenic potency of the 3 antigens used during this three-year period of study.

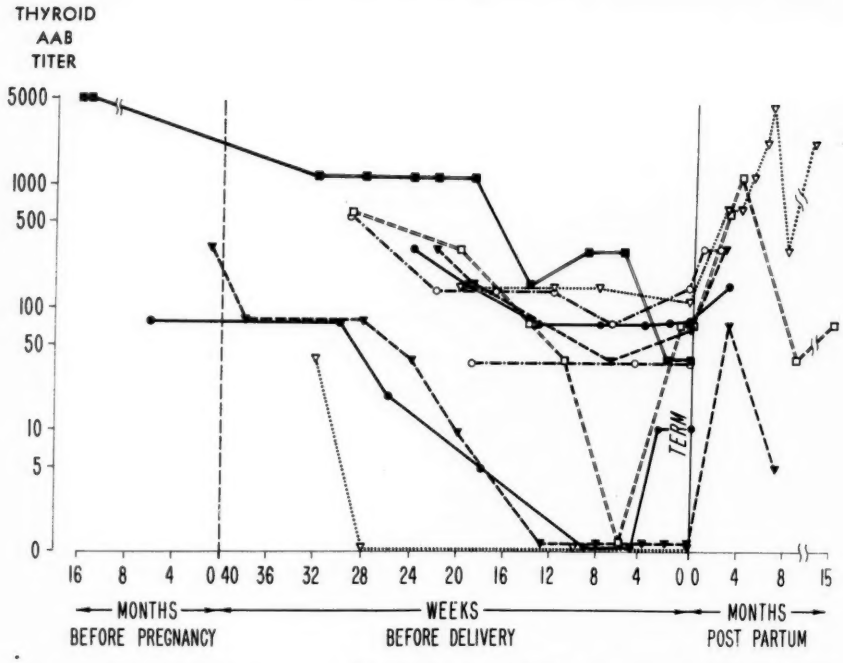

FIG. 1. Fall in maternal antithyroid antibody titers during pregnancy, and rise after termination of pregnancy.

RESULTS

Maternal AAB

A total of 22 women, 7 from the Clinical Radioisotope Unit and 15 from the 400 obstetrical registrants at Women's Hospital, found to have thyroid AAB either before or during pregnancy, were observed during 23 pregnancies. One patient aborted. Table 1 presents all pertinent data on these patients and their 23 pregnancies.

The AAB titers were quantitative in all serum specimens for 17 of these pregnancies. The serial AAB titers for the first 10 subjects in Table 1 are presented graphically in Figure 1. These women were followed to term with quantitative studies, and 2 or more AAB titers were determined during pregnancy with at least 1 positive result. AAB titers fell as early as the first trimester in all but 2 subjects (D.C. and C.M.) who were not tested until the second half of pregnancy when the titers in some of the other patients had reached a plateau. The titers in Patient D.C. rose after term, paralleling the rise observed in 6 of 7 patients followed after term. No positive titers were demonstrated in the seventh patient, who is included

in this report only because thyroid AAB had been demonstrated in her serum after the birth of a cretin six months before the present pregnancy. Three patients followed for more than six months after delivery showed a second fall in AAB after the postpartum peak.

Transplacental transfer

Blood samples were obtained at delivery on mother and child in 16 deliveries, as shown in Table 1. AAB was present in the maternal blood in 9 deliveries, at titers ranging from 10 to 160; AAB was demonstrated in the cord blood of 8 of these 9 infants. Fetal AAB titers were lower than maternal titers in 7 cases. There was no AAB in the cord blood of the infants delivered from mothers whose titers had fallen to zero. Retesting of the sera of 6 of the infants whose cord blood had contained AAB, showed that no AAB was present at the age of 1 month in 3 instances, at 2 months in 2, and at 3 months in 1, despite persistence of AAB in the sera of the mother.

Thyroid gland function of newborn infants

With the exception of Patient R.D.'s infant, who died at birth with erythroblastosis fetalis, and the aborted fetus of Patient E.A., all newborns appeared to be normal upon physical examination. All infants but one have now been followed for six weeks to two years and show no evidence of hypothyroidism.

Serum PBI levels in the cord blood of 6 newborns were normal. Later serum PBI levels in 4 of these infants, as well as in 2 others (one with, and one without transplacental transfer of AAB), were also within the normal range.

Incidence of demonstrable AAB in nonpregnant women

In 4 of 100 nonpregnant patients in the Medical Outpatient Clinic, matched for age with the obstetrical registrants at Women's Hospital, thyroid AAB was found in the serum. This incidence of 4 per cent was not significantly different from the incidence of AAB in obstetrical registrants at Women's Hospital.

DISCUSSION

Relationship of antithyroid antibodies to cretinism

We have confirmed Blizzard's observation that maternal AAB crosses the placenta but circulates in the infant's blood for only a brief period of time. Our data indicate that these antibodies disappear from the fetal circulation in less than four weeks. AAB was present in the circulation of 8 mothers throughout pregnancy and transmitted transplacentally to the

fetus (at least by term), but it was not a cause of cretinism. No cretin was born of 12 additional pregnancies in which AAB had been observed in the mother's serum prior to term but not at the time of delivery. It is apparent in our study, as in Blizzard's, that the presence of AAB in the maternal and the fetal circulations is not a cause of cretinism.

Decrease in AAB titers during pregnancy

The observed regular decrease in titers of circulating AAB during pregnancy and the rise after the termination of pregnancy apparently were not the result of administration of desiccated thyroid to the mothers, because the titers rose again after the termination of pregnancy while the medication was continued.

The decrease in titers during pregnancy could be attributed to at least two possible factors. The mother might have lost her circulating AAB to the fetus through transplacental transfer and union of AAB with the fetal thyroid gland. Against this possibility was the apparent absence of hypothyroidism in the newborn. However, fetal thyroid damage may have been produced that was insufficient to allow detection by the methods employed here.

A second factor in the decrease in antibody titers during pregnancy might be the hormonal change associated with the pregnant state. Increased estrogen production during pregnancy is an unlikely cause of a decrease in AAB, since in Patient D.C. the titer rose during the administration of estrogen, after a plateau following delivery. Increased production of chorionic gonadotropin, progesterone, or adrenocorticosteroids (5) during pregnancy conceivably could have an effect on antibody titer either through an *in vivo* action or a direct effect demonstrable by an *in vitro* technique. We are exploring these possibilities at present.

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BIOASSAY OF LONG-ACTING THYROID STIMULATOR (L.A.T.S.); THE DOSE-RESPONSE RELATIONSHIP

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ABSTRACT

The dose-response relationship for a long-acting thyroid stimulator (L.A.T.S.) is described, up to responses of 1,000. L.A.T.S. is a thyroid-stimulating material found in the blood of thyrotoxic patients. Its dose-response relationship differs from that of standard thyrotropin in a manner compatible with the concept that its action is similar to that of a low concentration of standard thyrotropin maintained for a long time.

A THYROID-STIMULATING agent which differs from thyrotropin (T.S.H.) is present in the serum of thyrotoxic patients (1–3). One of its differences from thyrotropin is that it accelerates thyroid secretion for a longer time after a single injection into an assay animal, a property which is probably a consequence of its longer half-life in circulating blood (4, 5).

In the past, this agent has been called, variously, "an abnormal thyroid-stimulating hormone" (1), "the thyroid activator" (4), and "the abnormal thyroid stimulator" (5). It is now called "long-acting thyroid stimulator," abbreviated as L.A.T.S. (6).

This paper describes two estimations of the dose-response relationship for long-acting thyroid stimulator. Blood samples from 2 thyrotoxic patients were studied separately. In addition, the dose-response relationship for U.S.P. Thyrotropin Reference Substance was determined. The assay method used was McKenzie's modification (7) of the method of Adams and Purves which utilizes plasma I^{131} measurements (8, 9).

METHODS

T.S.H. assay method

Preparation of the mice. Twenty-five weanling female mice were used for each assay. The animals underwent a three-week preparation period, during which time they were maintained with a low-iodine diet. During the first day of preparation they received 0.01 per cent methylthiouracil in their drinking water, and for the next ten days, 0.05 per cent. Then they were given plain water for ten days, after which they all received 1.5 μc. of carrier-free I^{131} intraperitoneally. Six to eighteen hours after the I^{131}, they all

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received 10 μg. of *l*-thyroxine intraperitoneally. Eighteen to twenty-four hours after the I^{131}, their drinking water was replaced by a solution containing a pancreatic digest of thyroid tablets at a concentration of 130 mg. of thyroid substance (B.P.) per 100 ml.

The radioactivity of each animal's thyroid was measured *in vivo* three days after the I^{131} injection. The mean values for the groups ranged from 35–60 per cent of the dose, averaging 50 per cent. Individual mice whose thyroids contained less than 15 per cent of the dose were rejected, but such animals were rare.

Bleeding and injection of the mice. Four days after receiving the I^{131}, the mice were bled from a cut tail vessel while being warmed in brass cylinders fixed on top of a brass tank containing water at 40° C. In this apparatus, the tails of the mice project through segments cut out of the rubber stoppers used to retain the mice in the cylinders. Immediately after being bled each mouse was injected, via a tail vein, with the material being assayed or with physiologic sodium chloride solution. The animals were bled again two hours after, and in assays of L.A.T.S. sixteen hours after, injection of the tail veins.

Counting the radioactivity of mouse blood. The mice were paired to enable the use of larger numbers without prohibitively increasing the time required for measuring the radioactivity of the blood samples. A 0.1-ml. sample of whole blood (0.05 ml. from each of the two paired mice) was pipetted onto a planchet on which lay a piece of lens paper moistened by 0.05 ml. of a sodium lauryl sulphate solution.[1] The lens paper and the sodium lauryl sulphate solution prevent the drying blood film from flaking off the planchet. Even with these precautions, new planchets do not hold down the blood films properly until they have been used once or twice. The blood films were counted beneath a shielded end-window Geiger tube (General Electric Corp., 2,B,7).

Responses. The response to the L.A.T.S. or to the standard thyrotropin injected was the difference between (a) the mean percentage change in the blood I^{131} level of the mice receiving the test material and (b) the mean percentage change in the blood I^{131} level of the mice receiving control injections of physiologic saline.

Determination of dose-response relationships

Long-acting thyroid stimulator. Two separate estimations of the dose-response relationship for L.A.T.S. were made, using serum of the 2 thyrotoxic patients described later. Multiple assays were performed in both instances, the results being combined. In each assay four doses of serum (0.3, 0.1, 0.033 and 0.011 ml.) were employed, as well as control injections of physiologic saline. The serial dilutions of the serum were made with saline, the injection volume always being 0.3 ml.

Standard thyrotropin. U.S.P. Thyrotropin Reference Substance was used. As with the L.A.T.S. assays, four doses of TSH were employed in each assay, together with control injections of saline. Multiple assays were performed, the results being combined. The upper and lower parts of the dose-response line were measured separately, doses of 0.9, 0.3, 0.1 and 0.033 mU being used in one series of assays, and doses of 8.1, 2.7, 0.9 and 0.3 mU being used in another series of assays.

Patients

Patient McCabe was a 48-year-old married woman suffering from untreated, mild, recurrent hyperthyroidism and severe exophthalmos. A subtotal thyroidectomy had been performed for thyrotoxicosis eighteen years previously. A nodule of regrown thyroid tissue was palpable in the region of the right lobe and isthmus. A radioiodine tracer test, performed shortly before the blood sample was taken, showed an 8-hour thyroidal I^{131} uptake of 74 per cent of the dose, falling to 61 per cent at 48 hours.

[1] Suggestion of Dr. Brown Dobyns.

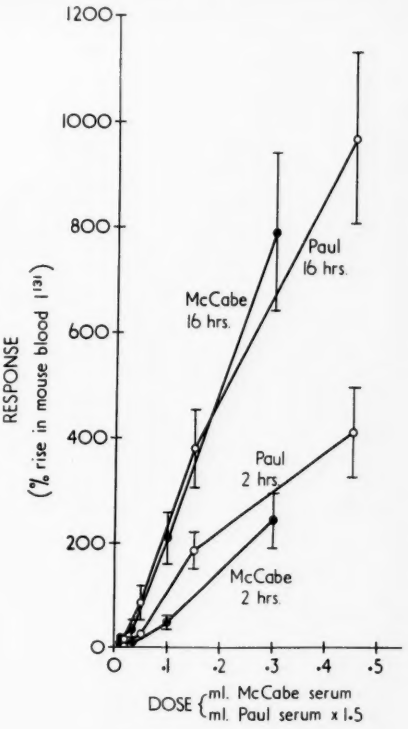

FIG. 1. The 2-hour and 16-hour responses elicited by various doses of the sera of 2 thyrotoxic patients, McCabe and Paul. The units on the dosage scale represent ml. of Patient McCabe's serum and ml.×1.5 of Patient Paul's serum. Each point represents the mean response of 16 to 20 mice. The limits of error are the standard errors of the means.

Patient Paul was a 33-year-old married man suffering from severe hyperthyroidism and slight exophthalmos. Prior to treatment, a radioiodine tracer test showed an 8-hour thyroid uptake of 73 per cent of the dose, falling to 44 per cent at 48 hours. Blood was taken when he was still hyperthyroid after having been treated with an inadequate dosage of carbimazole for several weeks. Subsequently, when receiving an adequate dosage, he showed a good response to this drug. After a year of medical treatment he was subjected to subtotal thyroidectomy. The excised tissue demonstrated uniform and marked hyperplasia.

RESULTS

Figure 1 shows the 2-hour and 16-hour responses elicited by various doses of serum from Patients McCabe and Paul. Each point on Patient McCabe's dose-response lines represents the mean response of 16–20 mice, and each point on Patient Paul's lines represents the mean response of 16 mice. The dose-response lines for the two sera did not differ significantly if it is assumed that the L.A.T.S. content of Patient Paul's serum was 1.5 times greater than that of Patient McCabe's serum.

Figure 2 shows the 2-hour responses elicited by various doses of standard thyrotropin (U.S.P. Thyrotropin Reference Substance). Each point represents the mean response of 28 mice. The upper and lower portions of the

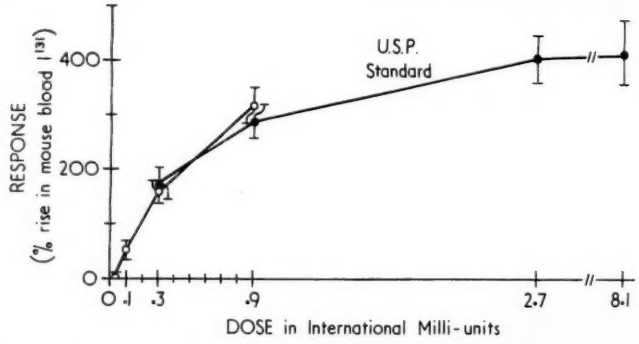

FIG. 2. The 2-hour responses elicited by various doses of U.S.P. Thyrotropin Reference Substance. In one series of assays the doses used were 0.033, 0.1, 0.3 and 0.9 mU and in a second series they were 0.3, 0.9, 2.7 and 8.1 mU. Each point represents the mean response of 28 mice. The limits of error are the standard errors of the means.

dose-response line were studied in separate series of assays, doses of 0.3 mU and 0.9 mU being included in both series.

In Figure 3 the responses are plotted against the logarithms of the doses. The 2-hour and 16-hour responses elicited by various doses of the sera of Patients McCabe and Paul are shown. The dosage scales of the two sera are aligned (as in Fig. 1) so that the doses of Patient Paul's serum correspond to 1.5-times-larger doses of Patient McCabe's serum. Single lines representing L.A.T.S. were fitted by eye through the 16-hour and 2-hour responses elicited by the two sera. A third line connects the points representing the 2-hour responses elicited by the various doses of U.S.P. Thyrotropin Reference Substance.

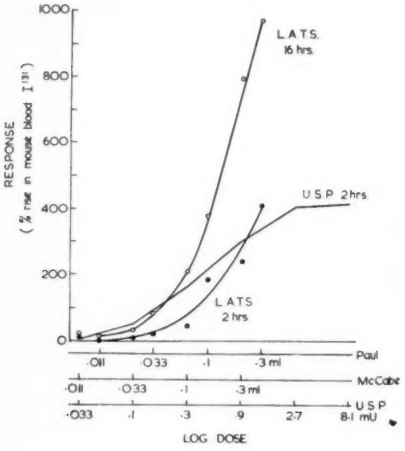

FIG. 3. The 2-hour responses (dots) and 16-hour responses (circles) elicited by the various doses of sera from Patients McCabe and Paul, plotted on a logarithmic dosage scale. Lines were drawn, by eye, through the points representing the 2-hour responses (L.A.T.S. 2 hrs.) and through the points representing the 16-hour responses (L.A.T.S. 16 hrs.). A third line shows the dose-response relationship of U.S.P. Thyrotropin Reference Substance (U.S.P. 2 hrs.).

It is seen that both the 16-hour and the 2-hour dose-response lines for L.A.T.S. differed in shape from the 2-hour dose-response line for standard thyrotropin. With doses eliciting responses greater than approximately 80, the L.A.T.S. dose-response lines were steeper than that of standard thyrotropin. Also, with standard thyrotropin the dose-response line became almost horizontal after the dosage reached 2.7 mU, when the responses were slightly over 400. The L.A.T.S. 16-hour dose-response line was still rising steeply when the response was nearly 1,000. The 16-hour responses to the two highest doses of L.A.T.S. were significantly greater ($P<0.001$, $P<0.01$) than the response to the highest dose of standard thyrotropin.

DISCUSSION

It is possible that long-acting thyroid stimulator is not a single substance, but varies somewhat in its composition from patient to patient. However, the present data provide no evidence for this, failing to show any difference between the L.A.T.S. from Patient McCabe and that from Patient Paul.

There is now clear evidence that L.A.T.S. is fully active in hypophysectomized mice (3, 10), and does not act on the thyroid indirectly by stimulating the pituitary to secrete thyrotropin. Presumably, therefore, it acts directly on the thyroid.

The data presented in this paper show that the dose-response line for L.A.T.S. is steeper than that for T.S.H., and that the maximal response attainable is much higher. These findings are in accord with the concept that the action of L.A.T.S. is similar to that of a low concentration of T.S.H. maintained for a long time. McKenzie (11) and Major and Munro (12) have shown that L.A.T.S. increases the thyroidal uptake of I^{131}, and they and other workers (13, 14) have shown that it alters thyroid histology similarly to T.S.H.

Major and Munro (12) found a very high level of L.A.T.S. in the blood of a thyrotoxic woman who had been treated by subtotal thyroidectomy. Injection of 0.5 ml. of her serum produced a thirtyfold increase in the mouse blood I^{131} level, the maximum response occurring thirty-six hours after the injection. When diluted 1 in 4,000, the serum still elicited a detectable response. These data can be combined with those presented in Figure 3 to give a further point on the 16-hour log dose-response line. The position of this point suggests that the line ceases to rise appreciably after the response has reached approximately 2,300. It appears that the serum from Major and Munro's patient contained approximately 200 times more L.A.T.S. than did Patient McCabe's serum.

T.S.H. can be recovered free of L.A.T.S. (15) if a mixture of the two is extracted by the alcohol percolation method of Bates, Garrison and

Howard (16). Another chemical difference is that L.A.T.S., in contrast to T.S.H., is poorly recovered after exposure to acetone (17).

The site of production of L.A.T.S. is still unknown, but there is now considerable evidence that it is not in the pituitary. Munro *et al.* (18) found no L.A.T.S., only T.S.H., in saline extracts of pituitary glands taken at autopsy from 2 thyrotoxic patients. Other evidence, less definite, is provided by the apparent occurrence of hyperthyroidism *a*) following presumably complete hypophysectomy (19), *b*) following pituitary stalk section which produced hypopituitarism (20), and *c*) in a patient with postpartum necrosis of the pituitary (21).

It is possible that the dose-response relationship for human thyrotropin differs from that for the currently used bovine standard preparation. The latter, therefore, may be unsuitable for use as a standard in assays of human thyrotropin. There is need for a human pituitary thyrotropin standard, as well as for a standard preparation of L.A.T.S.

Acknowledgments

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IODINE METABOLISM IN HASHIMOTO'S THYROIDITIS*

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ABSTRACT

A comprehensive study of iodine metabolism is reported in 40 cases of Hashimoto's thyroiditis. The results showed a dissociation between the mean absolute (or stable) iodine uptake by the thyroid (2.0 μg. per hour, which was normal) and the serum level of protein-bound iodine (2.5 μg. per 100 ml., which was significantly decreased). These findings indicate that the thyroid gland traps a normal quantity of iodine, but lacks the capacity to utilize it efficiently to form thyroid hormone. This faulty utilization of iodine is apparently a form of acquired dyshormonogenesis. Evidence of its nature is provided by the frequent discharge of iodine from the thyroid gland following administration of potassium perchlorate and by the presence of a butanol-insoluble iodinated protein in the plasma in many cases. The intrathyroidal exchangeable iodine was markedly reduced in almost all cases. This, and not the presence of the butanol-insoluble iodinated protein, explains the frequent discrepancy between the high level of PBI^{131} and the low level of PBI in serum. Standard radioiodine tests (thyroidal uptake and plasma activity) yield misleading results unless the foregoing abnormalities in stable iodine metabolism are taken into account.

ALTHOUGH the immunologic aspects of Hashimoto's thyroiditis have been extensively studied in recent years (1–5), comparatively little attention has been given to the mechanism of the disturbances in iodine metabolism which occur in this condition. The thyroidal uptake of radioiodine (I^{131}) has been reported as low (5–7), normal (5–10) or elevated (5–7, 9, 10), and the plasma protein-bound radioiodine (PBI^{131}) level as lying in the upper normal (11, 12) or the thyrotoxic range (9, 10, 13) even in the presence of clinical hypothyroidism (10). The results of standard I^{131} tests in this disease are often completely misleading, since in euthyroid patients with Hashimoto's thyroiditis the findings may be consistent with thyrotoxicosis (5, 10, 14). It has been suggested that the high levels of

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PBI^{131} reflect a rapid discharge of the isotope from a gland with a high turnover rate due to a small intrathyroidal iodine pool (9, 10, 12). Another possible explanation is suggested by the finding of Owen and McConahey (13) that an abnormal butanol-insoluble iodinated protein may be present in the plasma, at least in some cases.

In order to study iodine metabolism in Hashimoto's thyroiditis, we have applied a combination of isotopic and chemical techniques which we have previously used in the study of other thyroid disorders (15, 16). These techniques allow us to estimate the absolute amount of iodine taken up by the thyroid (AIU) in terms of micrograms per hour, and the intrathyroidal exchangeable stores of iodine in milligrams.

MATERIALS AND METHODS

Patients

Forty patients with untreated Hashimoto's thyroiditis were studied. Each patient had a firm goiter, either diffuse or nodular, and the diagnosis was confirmed in every case by positive results with the precipitin test (36 cases) and/or histologic examination of the gland (10 cases).

The precipitin test provides valuable confirmatory evidence of Hashimoto's thyroiditis (17). In our unit, 25 consecutive patients with goiter and positive precipitin findings underwent thyroidal biopsy. In 24 of the 25, the histologic features characteristic of Hashimoto's disease were present. In the remaining patient (Sjögren's disease) there were extensive Askanazy cell changes in the thyroid, but no round-cell infiltration. However, special care was taken to exclude subacute (granulomatous) thyroiditis both clinically and by radioiodine tests, because in granulomatous thyroiditis the precipitin test may yield temporary positive results (5). Cases of hypothyroidism without goiter but with positive precipitin findings were also excluded from the study.

Further evidence of Hashimoto's thyroiditis was provided by: 1) the thyroid complement-fixation test (18), for which results were strongly positive in 37 cases; 2) electrophoresis of serum proteins; and 3) the standard serum flocculation test, for which results were abnormal in 33 cases. Additional evidence was provided by the follow-up examination of the patients, and by observing shrinkage of the goiter during sodium thyroxine therapy.

There were 37 females with a mean age of 51 years (28 to 66 years) and 3 males with a mean age of 59 years (51 to 63 years). They were assessed clinically by the procedure described by Wayne (19), and were classified as euthyroid, equivocally hypothyroid or clearly hypothyroid. It was fully recognized, however, that such a distinction was arbitrary, as there was no sharp dividing line between these groups.

Methods

Standard radioactive iodine (I^{131}) tests, including 4-hour and 48-hour thyroidal uptake measurements and plasma PBI^{131} levels at forty-eight hours, were carried out by the methods described by Wayne (19, 20); PBI^{131} estimation was, however, simplified by using an ion exchange column (21). The potassium perchlorate discharge test (22) was performed as modified by Koutras *et al.* (16). A positive result was considered to be a fall in I^{131} uptake exceeding 10 per cent of the thyroidal radioactivity. Thyroxine suppression tests were performed by estimating the $2\frac{1}{2}$-hour thyroidal I^{132} uptake after administra-

TABLE 1. MEASUREMENTS OF STABLE AND RADIOACTIVE IODINE IN 40 CASES OF HASHIMOTO'S THYROIDITIS

Measurements	No. of cases	Mean ±S.E.	Range	Normal range
Thyroidal 2½-hr. uptake of I^{131} or I^{132} (% dose)	15	25.7 ±2.4	10.3 –46.6	10.0 –35.0
Thyroidal 4-hr. uptake of I^{131} (% dose)	40	37.0 ±2.2	7.5 –77.1	15.0 –45.0
Thyroidal 48-hr. uptake of I^{131} (% dose)	40	40.7 ±3.0	10.3 –79.8	20.0 –60.0
Thyroidal clearance (ml./min.)	16	22.7 ±3.7	2.9 –52.9	8.0 –40.0
Plasma inorg. iodine (PII) (μg./100 ml.)	15	0.20 ±0.04	0.02– 0.58	0.08– 0.60
Thyroidal absolute iodine uptake (AIU) (μg./hr.)	15	2.0 ±0.4	0.4 – 5.9	0.5 – 6.0
Plasma PBI^{131} (%/L.)	40	0.42 ±0.07	0.05– 2.74	0.0 – 0.40
Plasma butanol-insoluble I^{131} (% of PBI^{131})	9	31.0 ±9.0	0.0 –95.0	
Plasma PBI (μg./100 ml.)	39	2.5 ±0.2	0.5 – 5.3	3.2 – 7.2
Iodine utilization index	15	1.7 ±0.3	0.5 – 6.0	1.5 – 6.0
Intrathyroidal exchangeable iodine (mg.)	11	1.2 ±0.2	0.1 – 3.8	2.0 –18.0
Renal clearance of iodine (ml./min.)	15	28.1 ±1.9	16.5 –43.6	15.0 –55.0

tion of 0.2 mg. of sodium thyroxine daily for three weeks. The concentration of plasma PBI was measured by the method of Zak *et al.* (23) as modified by Farrell and Richmond (24). Thyrotropin (TSH) stimulation tests were performed in two ways. In 10 cases the 2½-hour thyroidal I^{132} uptake was estimated before and twenty-four hours after the administration of 10 U.S.P. units of TSH (Armour). In cases which did not respond by an increased uptake, another measurement was made after two further injections of TSH at 24-hour intervals (25). In 13 cases the plasma PBI^{131} level was measured before and twenty-four hours after a dose of 10 units of TSH, given nine days after a tracer dose of I^{131} (15, 16).

Estimations of the concentration of plasma inorganic iodine (PII), the absolute iodine uptake (AIU), and the renal radioiodine clearance rate were made by methods previously described (15, 16, 26). The intrathyroidal exchangeable iodine was calculated from the specific activity of the organic iodine discharged from the thyroid gland following administration of TSH (15, 16, 27).

The concentration of butanol-insoluble radioiodine was estimated on 5-ml. samples of plasma. The inorganic I^{131} was removed by an ion exchange resin column (21), and the precipitate remaining after butanol extraction was assayed for radioactivity.

RESULTS

Results of the studies of the metabolism of both stable and radioactive iodine are summarized in Table 1.

Thyroidal I^{131} uptake. In most cases the 2½-hour, 4-hour and 48-hour thyroidal uptakes of radioiodine and the thyroidal clearance rate lay within the normal range, but in several cases these values were above, and in a few cases below, the normal range; the standard deviation was therefore large. High values were found frequently for the 4-hour uptake, which exceeded the 48-hour uptake in 15 of the 40 cases. This is consistent with the short biologic half-life of radioiodine in Hashimoto's thyroiditis (9).

From the scattergram in Figure 1 it can be seen that, in general, the uptake of I^{131} was higher in the euthyroid patients than in those who were hypothyroid, but that there was a considerable overlap in the results.

Plasma inorganic iodine level. The PII values lay within the normal range, except in 2 patients for whom the values were 0.02 μg. and 0.06 μg. per 100 ml. respectively.

The absolute iodine uptake. The AIU was within the normal range, except in 1 hypothyroid patient (0.4 μg. per hour).

Plasma PBI^{131} level. The mean value for plasma PBI^{131} lay above the upper limit of normal and in 11 patients the value was in the thyrotoxic range. The PBI^{131} level tended to be higher in euthyroid subjects than in hypothyroid patients (Fig. 2). The percentage of the PBI^{131} which was not extractable by butanol varied from zero to 95 per cent (mean, 31 per cent). In 5 of 9 patients, the values for butanol-insoluble I^{131} exceeded 20 per cent of the total PBI^{131}.

Plasma PBI level. The PBI values lay in the hypothyroid or in the low-normal range (mean, 2.5 μg. per 100 ml., highest value 5.3 μg. per 100 ml.). The mean PBI level in the clinically euthyroid group (3.1 μg. per 100 ml.)

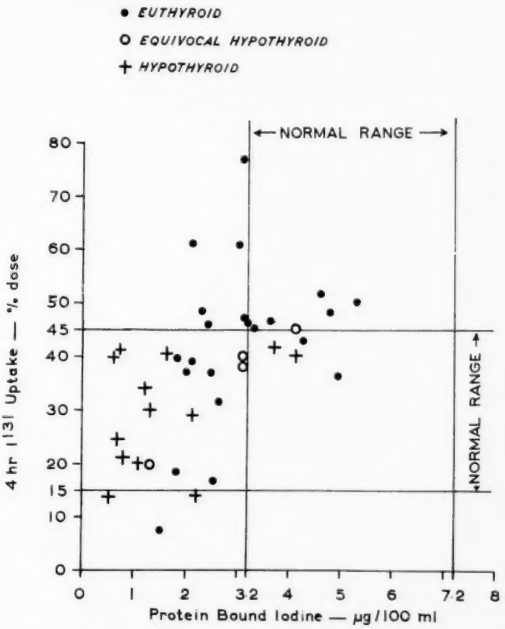

FIG. 1. Relation between 4-hour thyroidal uptake of I^{131} and plasma PBI concentration.

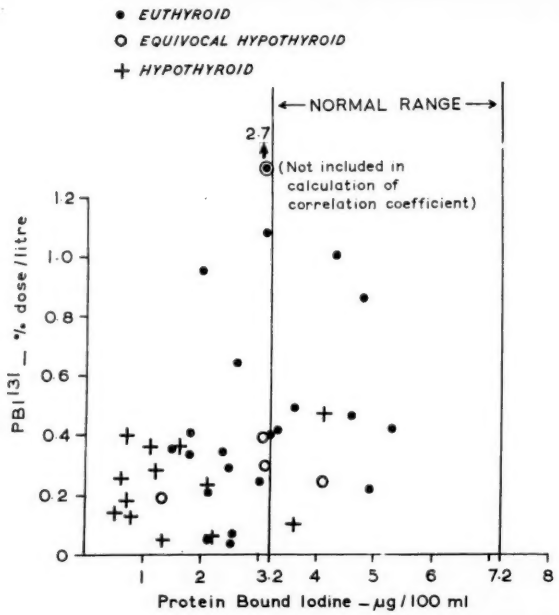

FIG. 2. Relation between PBI and PBI^{131} concentrations in plasma.

was significantly lower ($P<0.001$) than the mean normal value (5.2 µg. per 100 ml.) (Fig. 3). Confirmatory evidence of subclinical hypothyroidism in this group included: a low basal metabolic rate in 6 cases, a raised serum cholesterol level in 6 cases, and an electrocardiogram indicative of hypothyroidism in 4 cases. In Figures 1 and 2 it can be seen that the clinical

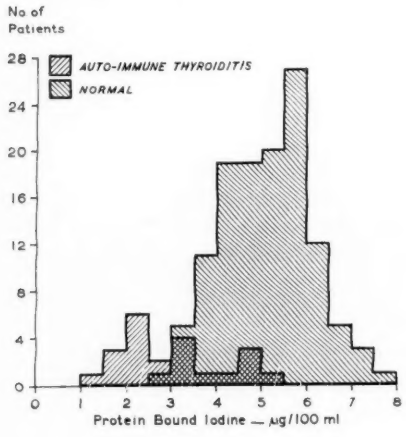

FIG. 3. Plasma PBI levels in euthyroid patients with auto-immune thyroiditis.

status agreed more closely with the plasma PBI level than with the PBI^{131} level or the 4-hour thyroidal I^{131} uptake.

Iodine utilization index. The ratio of PBI to AIU is a measure of the capacity of the thyroid gland to utilize the iodine presented, and we have termed it the iodine utilization index (16). In 6 of 15 patients with Hashimoto's thyroiditis the ratios were below the lower limit of normal. The mean of this index (1.7) was significantly lower ($P<0.01$) than the mean of our normal group (3.0); it tended to be lower in hypothyroid cases.

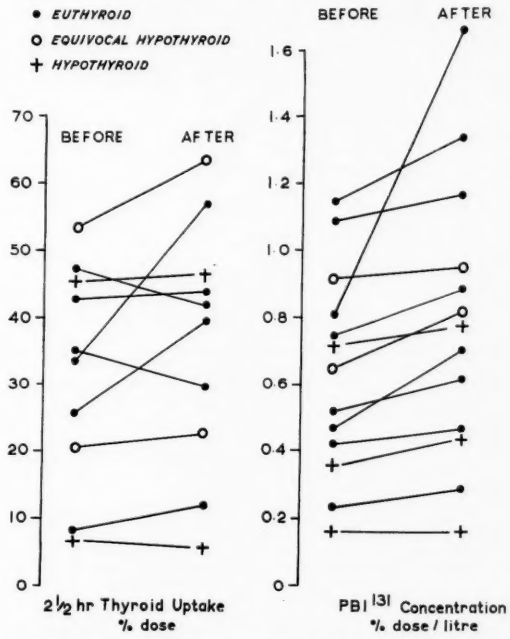

FIG. 4. Effect of TSH stimulation on thyroidal I^{132} uptake and PBI^{131} discharge.

The plasma PBI level was correlated significantly ($P<0.001$) with both the 4-hour thyroidal uptake of I^{131} ($r=+0.483$, Fig. 1) and the plasma PBI^{131} level ($r=+0.370$, Fig. 2).

Intrathyroidal exchangeable iodine. In 9 of 11 cases of Hashimoto's thyroiditis in which the intrathyroidal exchangeable iodine was calculated, abnormally low values were obtained. In 2 cases the discharge of protein-bound radioactivity after administration of thyroid-stimulating hormone (TSH) was insufficient to allow calculation of the intrathyroidal iodine; presumably in these 2 patients it was extremely low.

Renal clearance of iodide. The mean value for renal clearance of iodide

was within the normal range, although slightly lower than the mean of our normal group (34.0 ml. per minute). The difference, however, was not statistically significant.

Thyrotropin (TSH) tests. The results of the TSH tests are summarized in Figure 4. Of 10 cases in which the thyroidal I^{131} uptake was measured before and after administration of TSH, the uptake increased by 10 per cent or more in only 3 cases. The discharge of PBI^{131} after administration of TSH was measured in 13 cases during estimations of the intrathyroidal exchangeable iodine (27). All 8 euthyroid cases responded with a rise in protein-bound radioactivity, but in only 1 was the rise comparable with that found in normal controls. On the other hand, in only 1 of 3 hypothyroid cases was there a rise in the PBI^{131} level. All 34 cases tested showed normal suppressibility of the thyroidal uptake of radioiodine following administration of thyroxine.

Potassium perchlorate discharge test. This test yielded positive results in 12 of 27 patients. There was no correlation between the clinical status of the patient and the result of the test. The mean discharge in the positive cases was 17 per cent (maximum, 23 per cent).

DISCUSSION

Our patients with Hashimoto's thyroiditis had, as a group, a low concentration of plasma PBI, although their absolute (or stable) thyroidal iodine uptake (AIU) was normal. The low PBI level reflects decreased thyroid hormone production. The alternative explanation—increased peripheral degradation of thyroxine—is most unlikely, since the patients tended to be hypothyroid (28). Our results thus imply that the thyroid gland, in spite of retaining the normal amount of iodine, cannot utilize this iodide in the normal way to produce hormone. We have found the same pattern of iodine metabolism in our cases of congenital dyshormonogenesis (16), although the abnormality was more severe and the AIU was greater than normal. From the point of view of the handling of iodine, Hashimoto's thyroiditis may thus be considered as a form of "acquired dyshormonogenesis" in which the thyroid displays a diminished capacity for utilizing the iodine presented to it.

Other indices of iodine metabolism in Hashimoto's thyroiditis suggest that the defects in hormone synthesis are multiple. Thus a deficiency of organic binding of iodine is indicated by the discharge of iodine from the gland following the administration of potassium perchlorate (9, 10, 22), as observed in 12 of the patients we studied. Furthermore, in 5 of 9 cases the amount of abnormal butanol-insoluble iodinated protein exceeded 20 per cent of the total PBI^{131} concentration. Both abnormalities were present in some of our patients. Butanol-insoluble iodinated proteins have pre-

viously been reported in Hashimoto's thyroiditis (10, 13), congenital dyshormonogenesis (16, 29–31) and thyroid cancer (32, 33). Preliminary studies in 3 of our patients failed to show that this butanol-insoluble protein has the immunologic specificity of thyroglobulin (34). The possibility exists that our patients had dyshormonogenesis before the development of Hashimoto's thyroiditis; but this seems unlikely, since thyroid auto-antibodies are only rarely observed in congenital dyshormonogenesis (18).

In many cases of simple nontoxic goiter a discrepancy is found between the clinical status and the radioiodine uptake. In iodine-deficiency goiter the discrepancy is more apparent than real, since the high radioiodine uptake is not associated with a high AIU and can be explained on the basis of a dilution phenomenon (15). In such cases the actual amount of iodide retained by the thyroid (AIU) and the quantity of hormone produced are both normal. On the other hand, in congenital dyshormonogenesis and in Hashimoto's thyroiditis there is an actual dissociation between the amount of iodide retained and the amount of hormone synthesized. This explains a normal or even a high radioiodine uptake in a hypothyroid patient with Hashimoto's thyroiditis.

In almost all the cases of Hashimoto's thyroiditis which we have examined, the intrathyroidal exchangeable iodine was abnormally low. This is consistent with the reduction in biologic half-life of radioiodine reported by Doniach and Hudson (9). The PBI^{131} level is inversely related to the size of the intrathyroidal iodine pool (35). When this pool is small it may result in a high intrathyroidal turnover rate of iodine and a high PBI^{131} level, although the absolute amount of thyroid hormone released remains the same. This mechanism would account for the high PBI^{131} values observed in many of our cases. Since the proportion of butanol-insoluble material is the same, both in chemically determined PBI and in radioactive PBI^{131} (13), its degradation rate is presumably not much different from that of the normal hormone. It follows that the butanol-insoluble iodoprotein, although it contributes to the concentration of PBI^{131} in some cases, cannot account for the discrepancy between the high PBI^{131} and the low PBI values. This discrepancy can thus be explained by postulating that the high PBI^{131} level does not reflect an increased production of thyroid iodoproteins but a rapid turnover rate of iodine within the thyroid. The rapid turnover is probably the result of increased TSH stimulation. This is consistent with our finding of a poor response to exogenous TSH (Fig. 4), presumably because these cases are already under maximal or nearly maximal stimulation by endogenous TSH. The assumed increase in endogenous TSH can be attributed to the low level of circulating PBI, since the thyroidal radioiodine uptake is suppressed by administration of thyroxine.

The intrathyroidal exchangeable iodine is normal in cases of simple

nontoxic goiter (15, 36), but this test is too elaborate and time-consuming to be of practical help in the differentiation of simple goiter and Hashimoto's thyroiditis. Results of standard radioiodine tests vary markedly in Hashimoto's thyroiditis, but a pattern of some diagnostic value is the combination of a high plasma PBI^{131} level with either a normal thyroidal I^{131} uptake or a normal or low plasma PBI level. The potassium perchlorate discharge test yielded normal results in about half of our cases; nevertheless a positive result is diagnostically valuable. The finding of a low PBI level in many of our clinically euthyroid patients indicates an early degree of hypothyroidism, as previously noted by Skillern *et al.* (7).

The defects in iodine metabolism in Hashimoto's thyroiditis are essentially those which would be expected if a form of dyshormonogenesis occurred along with a decreased intrathyroidal exchangeable iodine pool. The standard radioiodine tests commonly used in clinical practice often yield abnormal results in this condition, but they can be explained if the whole picture of iodine metabolism is known.

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SYSTEM OF RADIOIODINE THERAPY FOR THYROTOXICOSIS AND NONTOXIC GOITER INVOLVING MEASUREMENT OF THYROIDAL RADIOSENSITIVITY*

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ABSTRACT

Measured thyroidal radiosensitivity factors were found to depend on goiter size but not on sex, thyroidal status (hyperthyroid or euthyroid), type of goiter (diffuse or nodular) or value of uptake rate. A method of radioiodine therapy for thyrotoxicosis is outlined which is predicted to yield a myxedema rate of about 2 per cent, with 60 per cent of the patients requiring only one dose, 30 per cent two doses, and 10 per cent three or more doses. The average time required to achieve a permanently euthyroid uptake rate is about ten weeks. Later than three years after completion of therapy there seems to be a slight fall in the uptake rate beyond that expected on account of the patient's aging.

INTRODUCTION

THE problem of determining the dose of radioiodine required to produce the desired effect in the treatment of thyrotoxicosis or nontoxic goiter has been investigated for some years. Linked with this is the problem of accurately assessing the effects of a given dose.

In the therapy of thyrotoxicosis, low myxedema and recurrence rates are sought. The maximal number of patients should be rendered euthyroid in the shortest possible time with the lowest possible dose of radiation.

The multiple small-dose technique results in an acceptable myxedema rate (1), but the prolonged treatment and the large number of doses involved are often unacceptable.

Empirical methods involving a lower average number of doses per patient and an acceptable duration of treatment, may produce myxedema in as many as 15 per cent of patients (2).

Methods employing mathematical formulae and aiming to administer

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a set radiation dose per gram of thyroid tissue (3, 4) are open to question because it is not proved that a given radiation dose per gram of thyroid tissue produces even approximately constant effects in all glands. In any case the planned dose of radiation is never accurately administered owing to uneven distribution of radioiodine throughout the thyroid (5, 6) and to the inevitable inaccurate estimation of mass (4). Myxedema develops in about 12 per cent of patients treated by this method (4).

The plan of treatment proposed here involves giving a first dose which is calculated on the basis of the maximal expected thyroidal radiosensitivity and is thus unlikely to cause myxedema. Data on radiosensitivity have been accumulated experimentally. Other quantities entering into the calculation of the first dose are: 1) the thyroidal radioiodine uptake rate in the untreated patient, and 2) the level to which this rate should be reduced. This level depends upon the patient's age and sex (7). The effect of the gland mass is included in the radiosensitivity data. A second dose of radioiodine, if necessary, is calculated on the basis of the patient's measured response to the first dose.

METHODS AND PATIENTS

Methods

Serial measurements of the thyroidal I^{131} uptake rate by the methods of Oddie *et al.* (8). were made on all patients in order to follow their thyroidal status as treatment progressed. These measurements gave the values of k_1 and k_2, the fractions of the inorganic iodide compartment that are cleared each minute by the thyroid gland and the kidneys respectively. Values of k_1 very soon after therapy were obtained by the method of Thomas *et al.* (9).

A quantitative measure of the thyroidal status before and at various times after treatment is provided by the value of k_1.

On the assumption (10) that the fraction of I^{131} retained from a therapeutic dose of D millicuries is the same as that from a preliminary tracer dose, the retained dose, d millicuries, is given by

$$d = \frac{k_1 D}{k_1 + k_2} . \qquad [1]$$

The patient's response as measured by the change in k_1 provides an index of the gland's sensitivity. A thyroidal radiosensitivity factor, L, is defined by the equation

$$Ld = \log_{10} \frac{k_1 \text{ before treatment}}{k_1 \text{ after treatment}} . \qquad [2]$$

This choice is empirical, but does give the correct answers for $d=0$ and $d \to \infty$. The selection of this formula is supported by our knowledge that $\log k_1$ is normally distributed for both euthyroid and hyperthyroid patients (7).

The size of the thyroid gland was assessed as 0, +1, +2, +3 or +4 as follows: 0, no enlargement of thyroid but isthmus palpable; +1, lateral lobes palpable; +2, moderate enlargement of the thyroid; +3, considerable enlargement; and +4, gross enlargement of thyroid.

TABLE 1. AVERAGE THYROIDAL RADIOSENSITIVITY FACTORS FOR A FIRST DOSE OF I^{131} IN THE TREATMENT OF GOITERS OF VARIOUS SIZES

Goiter size	No. of doses per patient used in the calculation of L	No. of cases	Sensitivity L		
			Mean	Individual standard deviation	Maximal L (1% limit)*
0	1	35†			
	>1	1			
	Any	36	0.157	0.125	0.448
+1	1	43‡	0.142	0.082	0.326
	>1	11			
	Any	54	0.121	0.085	0.319
+2	1	45	0.080	0.056	0.203
	>1	17			
	Any	62	0.063	0.055	0.192
+3	1	13	0.042	0.027	0.098
	>1	13			
	Any	26	0.028	0.031	0.100

* A one-sided percentage limit for the total cases, excluding any infinite values of L.
† One case (initially euthyroid) rejected with $L = \infty$.
‡ Two cases with $L = \infty$ rejected. One patient was obviously overdosed. The other was euthyroid and had just stopped taking antithyroid medication; the dose of I^{131} was based on a spuriously high initial uptake.

Patients

There were 124 patients with proven thyrotoxicosis (102 females and 22 males), 18 patients with unproven but probable thyrotoxicosis, and 39 euthyroid patients.

RESULTS

Sensitivity to a first dose

Patients in whom a thyroidal I^{131} uptake rate was measured before therapy and also ten weeks or more after therapy, were included in the analysis of thyroidal sensitivity to a first dose. Serial measurements during the first few weeks after therapy showed that the thyroidal uptake rate may either fall gradually, or fall to a low level and then revert to a higher stable level. This level is generally reached in eight weeks and always by ten weeks. The sensitivities were calculated with the use of the second equation. The mean values so obtained for each of the goiter sizes are shown in Table 1.

Distribution of sensitivity factors

The sensitivity factor, L, was found to be normally distributed. A chi-square test of goodness of fit for the distribution of L in the +1 goiter group, for example, yielded $P = 0.50$.

Statistically, it was not possible to demonstrate a difference in values of L between the proved hyperthyroid, unproved hyperthyroid, and euthyroid groups within each goiter size. Moreover, there was no correlation between the initial uptake rate and value of L either in the proved hyperthyroid group or the total group, when divided according to goiter size. Sex or type of goiter (diffuse or nodular) had no effect on L, nor did the

TABLE 2. THYROIDAL RADIOSENSITIVITY FOR REPEATED DOSES OF I^{131}

Patient	Sex	Goiter size or change in size	Radiosensitivity factors			L_2/L_1	Mean of L_2/L_1 and L_3/L_2
			L_1*	L_2	L_3		
L.R.	F	0	0.020	0.088	—	4.51	—
A.B.	F	0	0.069	0.218	—	3.18	—
D.I.	F	0	0.152	0.294	—	1.93	—
A.W.	M	0	0.033	0.166	—	5.06	—
E.B.	M	0	0.091	0.280	—	3.07	—
C.W.	F	+1	0.051	0.123	—	2.43	—
E.C.	F	+1→ 0	0.022	0.043	—	1.94	—
J.W.	F	+1	0.064	0.027	—	0.42	—
E.G.	F	+2	0.046	0.062	0.029	—	0.80
E.B.	F	+2→+1	0.064	0.168	—	2.61	—
M.A.	F	+2→+1	0.063	0.100	—	1.59	—
A.S.	F	+2	0.006	0.045	—	6.90	—
B.R.	F	+2→+1	0.026	0.136	—	5.19	—
A.H.	M	+2→+1	0.011	0.054	—	4.84	—
K.M.	M	+2→+1	0.043	0.092	—	2.13	—
W.I.	M	+2→ 0	0.025	0.100	—	3.97	—
E.C.	F	+3→+2→+2	0.025	0.010	0.126	—	2.27
U.J.	F	+3→+2	0.030	0.031	—	1.03	—
E.K.	F	+3→ 0→ 0	0.018	0.040	0.115	—	2.50
			L_4	L_5		L_5/L_4	
E.T.	F	+1	0.020	0.043	—	2.13	—

Let the ratio $r = L_n/L_{n-1}$; then mean $r = 2.93$
and r_{max} (1 per cent) $= 6.82$

* $L_n = \frac{1}{d_n} \log \frac{k_1 \text{ before } n\text{th dose}}{k_1 \text{ after } n\text{th dose}}$.

dosage of radioiodine. Consequently, goiter size alone was used to classify the data for calculation of L values.

Sensitivity to repeated doses

Patients on whom uptake rates had been measured ten weeks or later after a second or subsequent dose were included in an analysis of the relation between sensitivity to a first dose and that to a later dose. Patients with a negative L were excluded. In Table 2 are listed the sensitivity factors to second and first doses, and their ratio. Their mean ratio was 2.93, and a ratio of 6.82 was unlikely to be exceeded (1 per cent probability level).

Prediction of the lower L_1 values

Many patients whose responses to a first dose were inadequate were treated with a second dose sooner than ten weeks after the first, and consequently were not included in the first analysis of L_1 values. These rejected cases would have represented a higher proportion of low sensitivities and it was thought that the remaining populations would therefore be somewhat biased against low values. Information on the sensitivities in these cases was obtained as now outlined.

Let the sensitivity to the nth dose, d_n, of I^{131} be defined by

$$L_n d_n = \log \frac{k_1^{(n-1)}}{k_1^{(n)}} .$$

Then

$$\sum_{j=1}^{n} L_j d_j = \log \frac{k_1^{(o)}}{k_1^{(n)}} .$$

If

$$L_j = r L_{j-1} = r^{j-1} L_1$$

then

$$L_1 = \frac{\log \dfrac{k_1^{(o)}}{k_1^{(n)}}}{\sum_{1}^{n} r^{j-1} d_j} .$$

The retained doses, d_j, are known; $k_1^{(o)}$ is the untreated uptake rate; $k_1^{(n)}$ is the final rate after completion of therapy; and r is the ratio found to be 2.93. Thus the sensitivity, L_1, to the first dose may be calculated. These L_1 values have been added to Table 1.

Maximal likely L_1 for different goiter sizes

The values of L_1 (1 per cent) unlikely to be exceeded for each of the goiter sizes are shown in Table 1. They were not greatly affected by the inclusion of the lower sensitivities obtained by the multiple-dose analysis.

Radioiodine therapy in cases of intrathoracic goiter

The sensitivity factors for intrathoracic goiter are shown in Table 3. Goiters of +3 or +4 size in the neck and extending into the thorax had the lowest sensitivities in the whole series. In 16 of the 20 cases treated there was definite reduction in size of goiter, sometimes with softening of the gland; in 4 cases the size or hardness of the gland was not affected.

TABLE 3. AVERAGE THYROIDAL RADIOSENSITIVITY FACTORS FOR A FIRST DOSE OF I^{131} FOR PATIENTS WITH INTRATHORACIC GOITER

Goiter size in neck	No. of doses per patient used in calculation of L	No. of cases	Sensitivity L		
			Mean	Individual standard deviation	Maximal L (5 % limit)
0, +1 or +2	1	10	0.050	0.042	0.119
+3 or +4	1	5			
	>1	5			
	Any	10	0.007	0.014	0.029

Long-term trend of uptake rates in I^{131}-treated patients initially hyperthyroid

Thyroidal I^{131} uptake rates in both males and postmenopausal females are known to fall with age (7). This effect must be eliminated when searching for a possible long-term diminution in uptake after completion of radioiodine therapy for thyrotoxicosis. Uptake rates were measured on patients up to six years after therapy, and the values at these times were compared with the values at twenty weeks, working in terms of logarithmic rates that are known to be normally distributed (7).

The results are presented in Table 4. Three years or more after completion of therapy, there appears to be a fall in uptake greater than that expected on the basis of age. A larger series is being accumulated in order to assess this result with greater certainty.

TABLE 4. LONG-TERM CHANGES IN THYROIDAL I^{131} UPTAKE RATES AFTER I^{131} THERAPY FOR THYROTOXICOSIS

Weeks after I^{131} therapy	No. of cases	ΔZ*		
		Mean	Individual standard deviation	P†
30	76	−0.031	0.191	0.15
50	76	0.003	0.243	>0.90
80	49	0.002	0.226	>0.90
110	39	−0.010	0.294	0.85
150	15	0.178	0.223	<0.01
150–300 taken together	22	0.113	0.253	0.05

* The ΔZ values are the values of Z at twenty weeks minus the values at each specified time after therapy.

$$Z = \log \frac{k_1}{k_1^{(e)}}$$

where $k_1^{(e)}$ is the mean euthyroidal uptake rate for the patient's age and sex. ΔZ is thus a measure of any fall in k_1 over and above that expected on account of the patient's aging.

† The probability, P, that the observed difference from zero of the mean ΔZ is due to chance.

Proposed method of treatment of hyperthyroidism

Hyperthyroidism is diagnosed if a patient is clinically thyrotoxic and if either the thyroidal uptake rate before treatment lies in the clearly hyperthyroid range (7) or a suppression test yields a value in the hyperthyroid zone (11, 12). If radioiodine is the treatment of choice, the first retained dose is calculated by substituting in the second equation the maximal expected L for the size of the patient's goiter, the uptake rate k_1 before treatment, and the k_1 desired after treatment. This k_1 is the lower border of the clearly euthyroid range for the patient's age and sex (7). A dose of radioiodine calculated from the first equation is then administered.

If ten weeks later the patient is clinically thyrotoxic and either the uptake rate is still in the clearly hyperthyroid range or, though in the doubtful range, is clearly not suppressible (13), a second dose is given.

To calculate the second retained dose, the patient's actual sensitivity (L_1) to the first dose is calculated by substituting the first dose retained, the initial k_1 value, and the final k_1 value into the second equation. This L_1 is multiplied by 7 (1 per cent limit of r) and the second dose is calculated by substituting $7L_1$, the present value of k_1, and the k_1 desired after treatment, into the second equation.

A similar procedure is used to calculate any further necessary doses.

If a negative or zero result is obtained with the first dose, an arbitrary rule has been tentatively adopted of making the second retained dose three times the first.

Treatment by these methods is predicted, on the basis of data presented in this paper and elsewhere (7, 11, 12, 13), to yield a permanently euthyroid state by the use of one dose in about 60 per cent of patients. In 30 per cent of patients a second dose will be needed, and in 10 per cent, three or more doses. The myxedema rate should be 2 per cent and the recurrence rate near zero. A method substantially similar to that presented here, though not as accurate in detail, has been used for the past eighteen months. The results have been as follows: 50 per cent of cured patients received one dose; 38.5 per cent, two doses; and 11.5 per cent, three doses. Myxedema developed in 2 per cent of patients, and hyperthyroidism recurred in 2 per cent.

To achieve a permanently euthyroid state, the average retained total dose per patient is predicted to be about 8 mc., the average number of doses 1.5, and the average treatment period ten weeks.

Treatment of nontoxic goiter

Since it was not possible to demonstrate any difference between L values for hyperthyroid and euthyroid subjects, the system of dosage and the L

values used in the therapy of thyrotoxicosis are also used in the therapy of nontoxic goiter. In cases of nontoxic goiter the aim is to reduce the goiter size; therefore, the risk of a higher myxedema rate might be accepted in order to achieve a greater average reduction. The maximal L values used in the second equation can be adjusted (using the data in Table 1) to yield any required myxedema rate. In cases of intrathoracic goiter, toxic or nontoxic, the need for reduction in goiter size seems so urgent as to justify the acceptance of a 5 per cent myxedema rate after a first dose.

Potential of methods

It is agreed that the most well defined thyroid gland size is +1 goiter. It is noteworthy that the percentage standard deviation of sensitivity factors for this goiter size is the lowest. This suggests that a large fraction of the spread in the values of L is due to variation in gland mass. If the accuracy of methods for measuring gland mass could be improved, say by improved scintillation scanning techniques, then the mass could be written explicitly into the second equation, and a mass-independent sensitivity factor derived. The resulting sensitivity factors should be less dispersed and thus allow of more precise dosage. The average number of doses per patient and the duration of treatment would be reduced, and the occurrence of myxedema would be maintained at its present low value.

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COMPARATIVE EFFECTS OF ETHINYL ESTRADIOL, 17α-ETHYL-19-NORTESTOSTERONE AND METHYLTESTOSTERONE ON THE PLASMA CLEARANCE OF INFUSED CORTISOL

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ABSTRACT

Ethinyl estradiol, 17α-ethyl-19-nortestosterone or methyltestosterone in respective dosages of 1 mg., 100 mg. and 100 mg. daily were given for periods of ten to sixteen days to adult males convalescing from an orthopedic disease. Following the administration of ethinyl estradiol, nonconjugated plasma 17-hydroxycorticosteroid levels increased two- to threefold, but 17α-ethyl-19-nortestosterone and methyltestosterone were without effect. The plasma clearance of infused cortisol was decreased significantly in patients who received either ethinyl estradiol or 17α-ethyl-19-nortestosterone but was unaffected in patients given methyltestosterone. It is suggested that the inhibitory effect exerted by ethinyl estradiol on the removal of infused cortisol from plasma may be due to an increase in the plasma protein-binding of cortisol and also to a decrease in the hepatic catabolism of cortisol.

THE alterations which occur in the metabolism of cortisol during pregnancy and after the administration of estrogens have been of interest to several investigators (1–4). In these conditions, there is a distinct rise in the plasma cortisol level and a decrease in the rate of removal of infused cortisol from plasma (1–4). Evidence has been presented that these phenomena are related to an increase in the plasma protein corticosteroid-binding capacity and to a decrease in the hepatic catabolism of cortisol following increased circulating levels of estrogen (5–7). It was the purpose of this present study to compare the effects of ethinyl estradiol, 17α-ethyl-19-nortestosterone, and methyltestosterone on the rate of disappearance of intravenously administered cortisol from the plasma of hospitalized adult males.

MATERIALS AND METHODS

Twenty male patients, ranging in age from 23 to 65 years, were selected for study. All patients were undergoing an uneventful convalescence from an orthopedic disease, at least two weeks after operation. There was no clinical evidence of liver disease or endocrine disease.

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TABLE 1. EFFECTS OF ETHINYL ESTRADIOL, 17α-ETHYL-19-NORTESTOSTERONE, AND METHYLTESTOSTERONE ON PLASMA NONCONJUGATED 17-OH-CS LEVELS AND ON BROMSULFALEIN RETENTION

Subjects		Before hormone therapy		Duration of therapy (days)	After hormone therapy	
Number	Initials & age	BSP retention at 45 min. (%)	Plasma nonconj. 17-OH-CS (μg./100 ml.)		BSP retention at 45 min. (%)	Plasma nonconj. 17-OH-CS (μg./100 ml.)
		Ethinyl estradiol				
1	J.B., 23	3	20	14	6	53
2	N.C., 32	2	15	14	4	42
3	W.R., 46	5	17	14	10	65
4	T.A., 51	3	21	14	6	48
5	S.N., 65	4	14	14	5	57
	Mean		*17.4*			*53.0*
		17α-Ethyl-19-nortestosterone				
6	R.W., 35	2	15	12	25	18
7	G.Z., 43	5	18	11	32	24
8	E.B., 48	6	16	14	27	15
9	K.G., 49	4	19	10	30	12
10	H.M., 62	5	12	10	14	17
	Mean		*16.0*			*17.2*
		Methyltestosterone				
11	M.J., 36	3	23	12	16	20
12	R.B., 41	2	15	16	22	17
13	W.M., 43	4	12	14	14	14
14	P.R., 54	5	14	15	19	11
15	F.H., 62	2	19	14	11	19
	Mean		*16.6*			*16.2*
		Ethinyl estradiol and Methyltestosterone				
16	L.B., 29	1	23	10	35	45
17	J.S., 30	5	17	10	21	36
18	F.W., 34	3	18	14	28	51
19	F.F., 41	3	16	14	20	38
20	H.A., 64	6	20	10	37	54
	Mean		*18.8*			*44.6*

These patients were given cortisol (free alcohol) intravenously in a dosage of 1 mg. per kilogram of body weight. The cortisol was dissolved in 0.85 per cent sodium chloride solution and infused over a thirty-minute period. Blood samples were drawn before, and at one, two, four and six hours after the beginning of the infusion.

Following this test, ethinyl estradiol was given orally to 5 patients in a dosage of 1 mg. daily (0.5 mg. every twelve hours). Five patients received orally 100 mg. of 17α-ethyl-19-nortestosterone (ENT) daily (50 mg. every twelve hours). Methyltestosterone was administered orally to 5 patients in a dosage of 100 mg. daily (50 mg. every twelve hours). In an additional 5 patients, both ethinyl estradiol (0.5 mg. twice a day) and methyltestosterone (50 mg. twice a day) were given daily by mouth. All these hormones were administered for periods varying from ten to sixteen days (Table 1).

Each patient received a standard cortisol infusion on the first morning following the last day of hormone administration. The concentration of nonconjugated plasma 17-

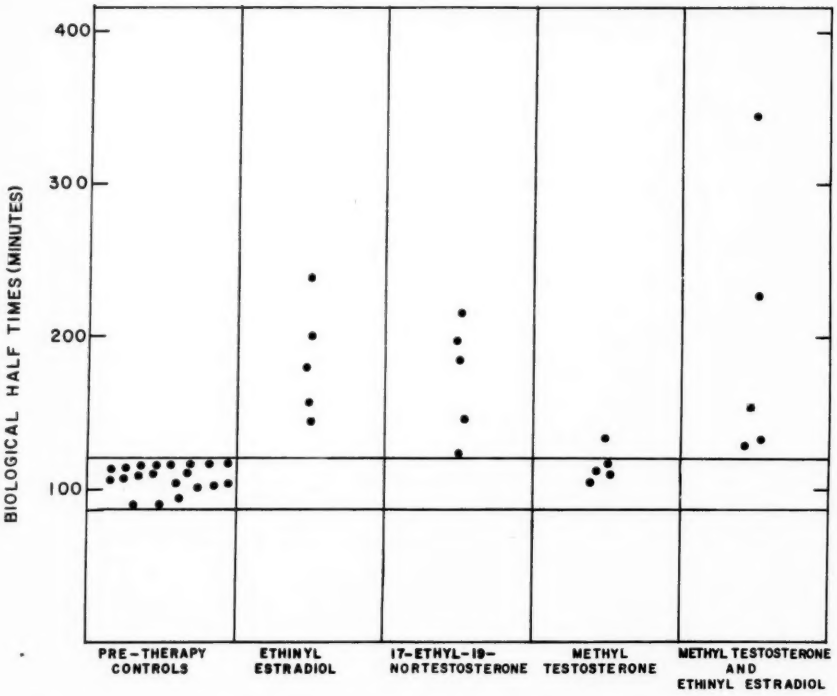

FIG. 1. Comparative effects of ethinyl estradiol, 17α-ethyl-19-nortestosterone, and methyltestosterone on biologic half-times of intravenously administered cortisol in normal subjects.

hydroxycorticosteroids (17-OH-CS)[1] was determined by the method of Nelson and Samuels (8). Liver function was evaluated by measuring bromsulfalein (BSP) retention at forty-five minutes, using 5 mg. per Kg. of body weight (9), both before and after hormone therapy.

RESULTS

Effects of ethinyl estradiol, 17α-ethyl-19-nortestosterone (ENT) and methyltestosterone on plasma nonconjugated 17-OH-CS levels and on BSP retention

Administration of ethinyl estradiol for two weeks resulted in a marked rise of the plasma nonconjugated 17-OH-CS level in all patients, but an increase in BSP retention in only 1 patient (Table 1). ENT and methyltestosterone were without effect on the plasma level of nonconjugated

[1] The term nonconjugated plasma 17-OH-CS is used in preference to free plasma cortisol for two reasons: 1) the major part of these compounds are bound to plasma proteins (15), and 2) following the infusion of pharmacologic doses of cortisol, only about two-thirds of the nonconjugated plasma 17-OH-CS consist of cortisol itself (18).

17-OH-CS, but caused a significant increase in BSP retention in every patient (Table 1). The combination of ethinyl estradiol and methyltestosterone in the dosages used in this study produced an elevation of plasma nonconjugated 17-OH-CS levels and also a marked increase in BSP retention (Table 1).

Effects of ethinyl estradiol, ENT and methyltestosterone on the plasma clearance of infused cortisol

In Figure 1, the biologic half-times of infused cortisol[2] are compared both before and after the completion of hormone therapy in all patients. Before hormone administration, the mean biologic half-time of cortisol disappearance from plasma was 114 minutes with a range of 93 to 128 minutes in these 20 subjects. Ethinyl estradiol produced a prolongation of the biologic half-time of cortisol in all 5 patients, ranging from 142 to 240 minutes. Administration of ENT resulted in an elevation of the biologic half-time of cortisol in 4 of 5 patients, ranging from 143 to 214 minutes. Essentially normal values were found in the 5 patients who received methyltestosterone.

The combination of ethinyl estradiol and methyltestosterone exerted varying effects on the rate of disappearance of cortisol from plasma. No significant change occurred in 2 patients. However, the biologic half-times increased to 231 and 349 minutes in 2 subjects in whom nausea, vomiting and right upper quadrant distress developed while they were receiving both hormones. These symptoms disappeared promptly after the cessation of therapy. One other patient who demonstrated a moderate elevation of the biologic half-time of cortisol remained asymptomatic.

DISCUSSION

It appears that the administration of either ethinyl estradiol or 17α-ethyl-19-nortestosterone (ENT) to adult males produces significant alterations in the plasma clearance of infused cortisol. Our results with ethinyl estradiol are in agreement with those reported by numerous investigators (2, 4, 7).

The disappearance of infused cortisol from plasma depends first upon its distribution between the circulating blood, the extracellular fluid, and the body tissue steroid pool (10, 11), and second upon its metabolic disposal, primarily by the liver (11, 12). Renal excretion of cortisol does not

[2] The use of biologic half-times of cortisol, derived from semilogarithmic plots, as quantitative figures has the theoretical objection that the system being studied does not really behave as a single compartment-exponential disposal rate system. With this limitation in mind, the comparative biologic half-times of cortisol may be considered as a satisfactory way in which to indicate a qualitative difference.

play a significant role in the regulation of plasma nonconjugated 17-OH-CS levels (10). The distribution of cortisol between the intravascular compartment and the extracellular fluid may be influenced by the ability of the plasma proteins to bind cortisol (13, 14). Various workers have demonstrated the existence of a relatively specific corticosteroid-binding protein in human plasma (13, 15). The corticosteroid-binding capacity of plasma is greatly increased during pregnancy and also during the administration of estrogens (5, 6, 15).

Mills *et al.* (16) have suggested that the elevated plasma nonconjugated 17-OH-CS values observed during estrogen therapy are related primarily to an increase in the protein-bound cortisol fraction. It seems logical to assume that plasma protein binding of cortisol protects the circulating hormone to some extent from catabolism by the liver. This concept could explain the decreased rate of removal of infused cortisol from plasma, but it does not satisfactorily account for the following observations: 1) during administration of ethinyl estradiol the plasma clearance of tetrahydrocortisone is slower than in control subjects (6), and 2) the rate of formation of plasma conjugated 17-OH-CS following the infusion of cortisol may be decreased during estrogen therapy (7).

These phenomena can be explained by the thesis that increased circulating levels of estrogen can influence the rate of cortisol metabolism by the liver. Troop (17) showed that the daily injection of estradiol-17β to gonadectomized rats decreased the capacity of their liver homogenates to reduce the 17,21-dihydroxy-20-ketone side-chain of cortisone, whereas testosterone-treated animals showed an increased ability to metabolize the same side-chain. Whether administration of ethinyl estradiol to man can alter the capacity of his hepatic steroid reductases to metabolize cortisol awaits definitive proof.

ENT therapy did not affect the plasma nonconjugated 17-OH-CS levels in our patients. This result furnishes indirect evidence that ENT does not increase the plasma protein cortisol-binding capacity in man. However, the mechanism by which ENT may prolong the biologic plasma half-time of infused cortisol is not apparent from our study.

It is of interest that methyltestosterone did not alter the plasma clearance of infused cortisol, for an increase in BSP retention occurred in all patients who received this hormone. This observation suggests that drugs which can increase BSP retention in man may not necessarily affect the ability of the liver to metabolize cortisol.

In the dosages used in this study, methyltestosterone did not prevent ethinyl estradiol from raising the plasma levels of nonconjugated 17-OH-CS. It was thought originally that methyltestosterone might neutralize the inhibitory effect of ethinyl estradiol on cortisol metabolism in man; indeed

in 2 of our patients there was no significant impairment in the clearance of infused cortisol from plasma while they were receiving both hormones. However, the administration of these hormones together to 2 other patients resulted not only in a marked increase in BSP retention and in the biologic plasma half-times of infused cortisol but also in the appearance of nausea, vomiting and abdominal distress. The possibility that the combination of methyltestosterone and ethinyl estradiol may prove toxic to the liver in certain patients caused us to discontinue this medication.

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PRIMARY HYPERPARATHYROIDISM ASSOCIATED WITH A CYSTIC LESION IN THE NECK: PROBABLE PARATHYROID CYST

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ABSTRACT

A case of primary hyperparathyroidism is described in which there was clinical and biochemical resolution after a cystic lesion in the superior left lobe of the thyroid gland was surgically removed. On histologic step-section of the cyst an aggregation of epithelial cells was seen in its wall but they could not be definitely identified as parathyroid in origin. Clinical and biochemical evidence is presented which suggests that the lesion was a hyperfunctioning parathyroid cyst. Emphasis is placed on careful historical evaluation regarding manifestations of hyperparathyroidism in patients who have a mass in the anterior neck. Appropriate laboratory investigation in suspected cases may help to document the functional significance of such lesions.

TWENTY-SIX cases of macroscopic cysts of the parathyroid glands have been recorded by various observers (1–7). The lesions have been distinguished from other cysts of the neck by their anatomic location and the histologic demonstration of aggregations of parathyroid cells in the walls of the cysts, or occasionally by only a single lining layer of cuboidal or columnar cells (5).

Examination of these parathyroid cysts has been occasioned by the excison of what was preoperatively thought to be an asymptomatic mass in the anterior neck or a solitary nodule of the thyroid gland. Therefore a preoperative endocrinologic study of these patients had seemed unnecessary, and it had been assumed that the cysts were in no way associated with dysfunction of the parathyroid glands. Two of the patients reported by Welti (7), however, complained of weakness, and one of them exhibited decalcification of bone.

The present report concerns a patient who had the metabolic features of primary hyperparathyroidism associated with a palpable nodule in the region of the left lobe of the thyroid gland. Pathologic examination of the excised specimen revealed a cystic structure without recognizable para-

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thyroid cells. Prompt disappearance of the clinical and biochemical derangements of hyperparathyroidism was noted postoperatively.

CASE REPORT

Preoperative course

A 69-year-old Caucasian woman of German extraction was well until the age of 52 years (1943), when she was admitted to the Hospital for Mental Diseases because of manic depressive psychosis. She remained there for seventeen years. In July 1950, enlargement of the posterior neck and apparent weakness of the muscles of the cervical spine prompted roentgenographic examination, which showed an old compression fracture and dislocation of the body of the seventh cervical vertebra. Except for this x-ray finding, chronic constipation, and gradually progressive paralysis agitans, her general health was good until February 1956, when she suffered fever and dysuria. Urinalysis revealed many white blood cells per high-power field. Intravenous pyelography showed

TABLE 1. PREOPERATIVE AND POSTOPERATIVE STUDIES OF PARATHYROID FUNCTION

	Days before surgery				Days after surgery							
	35	32	17	12	1	2	3	4	16	30	118	120
Serum calcium (mg./100 ml.)	13.6	14.0	12.7	11.9	9.2		7.7	8.6	10.1	10.0	10.1	10.6
Serum phosphorus (mg./100 ml.)	3.1	2.82	2.4	3.1	2.74		2.74	3.9	3.9	3.2	3.2	3.2
Urinary calcium* (mg./24 hrs.)	250		213			110	86			11	61	
Tubular reabsorption of phosphorus (%)	83									86		89
Plasma calcium fractionated** (mg./100 ml.)	Total, 12.7 Diffusible, 7.62 (60%) Nondiffusible, 4.58 (40%)											
Serum alk. phosphatase (Bessey-Lowry units)	3.15				3.45							

* Determinations performed both during and after a three-day period of 150-mg. calcium diet.
** Method of Breen and Freeman (8); by this method, 53 per cent of the total plasma calcium is normally diffusible.

a normal-appearing left kidney and multiple calyceal calculi of the right kidney without obstructive uropathy. The serum calcium, inorganic phosphorus and alkaline phosphatase levels were not determined at this time.

In January 1958, there was a recrudescence of pyelonephritis, and a right nephrectomy was performed. Multiple calcium oxalate stones were discovered in the pelvis of the removed kidney.

In March 1958, the patient underwent cholecystectomy for chronic gallbladder disease. At this time no masses in the anterior neck were recorded as palpable. The serum alkaline phosphatase level was slightly elevated (average, 5.0 Bessey-Lowry units). The serum bilirubin concentration and the results of other tests of liver function were within normal limits. The serum nonprotein nitrogen level was 35 mg. per 100 ml.

The patient came to our attention in March 1960, when she was referred for evaluation of mild hypertension. She exhibited moderate mental depression, slow cerebration, poor memory, and severe classic parkinsonism, all of which did not lend reliability to her history. She did, however, complain of polyuria, the onset of which could not be determined accurately. The staff of the Hospital for Mental Diseases had noted progressive weakness and lethargy. She had been able to perform light work and was ambulatory until the week before admission, when she could no longer get out of bed unaided. There was no history of excessive intake of alkali or vitamin D.

Pertinent physical findings included a blood pressure of 200 mm. Hg systolic and 85 mm. diastolic, a soft nodule 1 cm. in diameter in the region of the left lobe of the thyroid, symmetrically hypoactive deep tendon reflexes, and surgical scars corroborating the stated surgical history.

The relevant laboratory data are summarized in Table 1. The hemoglobin level and white blood cell count were normal. Urinalysis showed a specific gravity of 1.017, acid reaction, a trace of protein, and 10–20 white blood cells per high-power field. The concentration of fasting blood sugar was 95 mg. per 100 ml. Results of a serologic test for syphilis (Kline) were negative. The plasma carbon dioxide content, and levels of serum sodium, chloride and potassium were 22.9, 138.0, 105.4 and 4.8 mEq. per liter, respectively. Plasma urea nitrogen concentration averaged 19.7 mg. per 100 ml. (upper limit of normal, 22.0 mg.). The serum protein electrophoretic pattern was within normal limits. Proteus and Pseudomonas aeruginosa were present in significant numbers in a urine culture.

X-ray examination of the chest revealed a sclerotic aorta, but the bony structures, lungs, and heart were within normal limits. Fluoroscopic and cineradiographic study of the barium-filled esophagus showed no defect consistent with a parathyroid adenoma. Intravenous pyelography demonstrated a normal outline of the left upper urinary tract. Roentgenograms of the skull, spine, pelvis, long bones, and hands showed no evidence of demineralization or metastatic neoplasm. No myeloma or neoplastic cells were demonstrated on bone marrow examination. The electrocardiographic Q-T interval was 0.40 second at a cardiac ventricular rate of 60 per minute.

On April 11, 1960, the anterior neck was explored by Dr. Leonard Worman. The structures were easily visualized, and both lower parathyroids and the right upper parathyroid were identified; they appeared normal. A biopsy specimen was taken from the right lower parathyroid; the others were left intact. In the left upper and posterior aspect of the thyroid gland was a spherical cystic lesion measuring 1.5 cm. in diameter. It was well encapsulated and was shelled out easily from the surrounding thyroid tissue.

Pathology

Dr. Paul Kimmelstiel examined the lesion. His report was as follows:

"The specimen was a cyst measuring 1.5 cm. in diameter, the external aspect of which was bluish red. It contained a golden brown-yellow pasty material without odor. Culture of this material for fungi, tubercle bacilli, and bacteria showed no growth. The wall of the cyst was 3 mm. in thickness.

"Histologic step sections were made of the entire cyst. They showed a cyst lined by dense collagenous connective tissue within the thyroid gland. The small amount of surrounding thyroid tissue showed areas suggestive of involuting hyperplasia. Toward the luminal side, the cyst wall revealed focal accumulations of small round cells and histiocytes with abundant foamy cytoplasm, cholesterol crystals and occasional foreign body giant cells. Parathyroid tissue could not be definitely identified (Fig. 1, a and b). The grossly normal parathyroid gland, separately submitted, was histologically normal."

Postoperative course

The patient's postoperative course was uncomplicated. There were no clinical signs of hypoparathyroidism at any time, even though a temporary depression of the serum calcium level was observed. She became unquestionably more alert and physically active, and was less depressed. She vividly recalled past events which she could not remember prior to surgery. Deep tendon reflexes became more brisk and bowel function im-

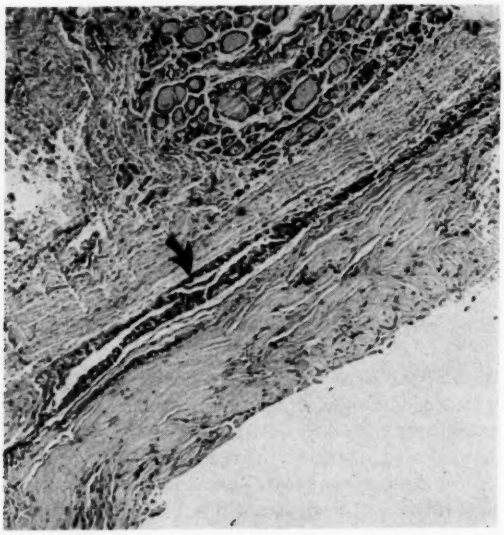

FIG. 1a. Wall of the cyst containing a linear collection of cells with epithelial features. Thyroid tissue lies above, opposite the luminal aspect of the cyst (reduced from ×100).

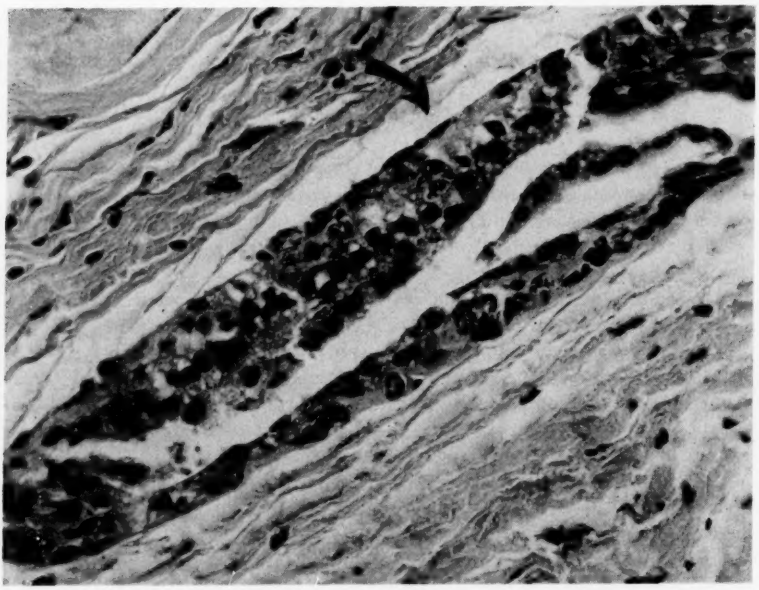

FIG. 1b. More detailed view of cells with epithelial features (×420).

proved. General clinical improvement has been maintained during the four-month period following surgery.

DISCUSSION

It is difficult to challenge the diagnosis of primary hyperparathyroidism in this case (Table 1). Psychosis, renal stones, striking weakness and polyuria were important features of the patient's illness. Preoperatively the serum calcium level was persistently elevated and the serum phosphorus level was at the lower limits of normal on two occasions. Both the total diffusible plasma calcium and the percentage of diffusible plasma calcium were increased. A similar change in the distribution of the plasma calcium was reported by Lloyd and Rose (9) in 16 of 17 patients with hyperparathyroidism.

Although the group of cells shown in Figure 1 could not be identified as parathyroid cells with any degree of certainty, they had epithelial features and did not resemble the cellular tissue of the thyroid gland. Perhaps these cells represented the hyperfunctioning remnants of a parathyroid cyst which had undergone avascular degeneration. This concept might be supported by the slight decrease in the serum calcium level observed during the weeks prior to surgery. The temporary fall in the concentration of serum calcium to subnormal levels in the immediate postoperative period and the return to normal further suggest that the removed lesion was previously hyperfunctioning. That normally functioning parathyroid tissue was still present in this patient is supported by the maintenance of physiologic serum calcium levels during the four-month postoperative period. It is most unlikely that the effect produced by the removal of one normal parathyroid gland or the operative trauma sustained by the remaining normal parathyroid glands could explain the observed clinical and biochemical improvement.

To our knowledge, this case is the first to be reported of a patient with a macroscopic cyst of the neck in which parathyroid tissue could not be definitely demonstrated and in whom the metabolic features of primary hyperparathyroidism have been documented. Greene and associates (10) described the only reported case in which functional features were ascribed to a microscopically proved parathyroid cyst; typical temporary hypoparathyroidism developed after excision of the lesion.

Our case emphasizes the importance of a careful search in the history for the vague symptoms and diffuse manifestations of primary hyperparathyroidism in patients who have a mass in the anterior neck. When the history indicates the possibility of hyperparathyroidism, serum calcium and phosphorus levels should be determined as a screening measure (11). In this way the functional significance of nondescript lesions such as the

one described, and of occult parathyroid neoplasms removed incidentally, will be definitively documented.

Acknowledgments

We are indebted to Dr. Smith Freeman, Chairman of the Department of Biochemistry, Northwestern University, for kindly performing the serum protein-bound and unbound calcium determinations. We thank Mr. Anthony M. Kuzma, photographer, Marquette University School of Medicine, for the photomicrographs. The advice and help of Dr. Paul Kimmelstiel, Chairman of the Department of Pathology, Milwaukee County Hospital, is appreciated.

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STUDIES ON THE BIOLOGIC CHARACTERIZATION OF HUMAN GONADOTROPINS. VII. URINARY GONADOTROPINS OF MEN, POSTMENOPAUSAL WOMEN AND EUNUCHS*

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ABSTRACT

Human pituitary gonadotropin (HPG) was prepared by the kaolin-acetone method from large quantities of urine of normal men, postmenopausal women and eunuchs. These 3 preparations of HPG were studied by a general gonadotropin assay, by assays for FSH and LH, and by the fingerprint system for identification of gonadotropins. The ratio of gonadotropic potency of the HPG preparations from men and postmenopausal women was the same regardless of the methods of assay used, indicating that the proportions of FSH and LH in the HPG from these two sources were the same. However, the HPG from the urine of eunuchs contained more FSH than LH as compared with HPG from the urine of men or postmenopausal women. The FSH/LH ratio in HPG from eunuchs was about twice that of HPG from men or postmenopausal women.

ALTHOUGH the biologic nature of the gonadotropin found in the urine of nonpregnant human beings (human pituitary gonadotropin, or HPG) is still uncertain, most workers regard it as a mixture of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In addition to the reported partial chemical separation of two principles, this concept is supported by the recent findings with certain biologic assays that the urine of men contains more LH than does the urine of postmenopausal women and that the latter contains more FSH than does the urine of men (1). However, other evidence obtained by similar, although not identical, biologic assays indicates no difference in the content of FSH and LH in the urine of men and postmenopausal women (2). Thus, the dissimilarity of HPG, which constitutes a major biologic support for the concept of its dual nature, is not firmly established.

The conflict in the evidence appears to reside in the specificity of the biologic assays used. Both groups of workers employed general gonadotropin assays and an "LH" assay but did not include an assay currently

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acceptable as reasonably specific for FSH. Consequently, it was thought necessary to re-examine this problem. In the present study, we have, as in the past, used a general gonadotropin assay to determine quantitatively the differences in potency of HPG from various sources. With this as a guide, we then employed three assays designed to detect qualitative differences in the constitution of gonadotropin. These three assays were: 1) the fingerprint assay, which is presumably capable of reflecting large differences in the composition of gonadotropin, 2) the weight of the ventral prostate for estimation of "LH," and 3) ovarian augmentation for estimation of "FSH." In addition to HPG from the urine of men and postmenopausal women, a third source of HPG, namely the urine of eunuchs, was included.

MATERIALS AND METHODS

Preparation of HPG extracts

A batch comprising 60 liters of urine was adjusted to pH 4.5 with glacial acetic acid and stirred. This batch then was divided into four 15-liter portions, each of which was placed in a plastic vessel. To each of the four plastic vessels, 75 Gm. of kaolin suspended in 300 ml. of tap water at pH 4.5 was added. The urine was stirred and the kaolin allowed to settle overnight. The next morning, the supernatant urine was siphoned from each vessel. One 50-ml. beakerful (11 Gm.) of dry celite was added to the kaolin in each vessel, with stirring. The contents of one vessel was run through an automatic filter, and the residual urine was discarded. The celite-kaolin cake then was washed with 2 liters of tap water containing 1 ml. of glacial acetic acid (pH 4.5). The wash was discarded. The cake from the filtration chamber was removed and mixed in a Waring blendor with 200 ml. of fresh 2*M* ammonium hydroxide for one minute. The filtration chamber was precoated with half a beakerful of celite, and the contents of the blendor was poured into the chamber and filtered. The filtrate was saved. The celite-kaolin cake again was removed from the chamber and mixed in a Waring blendor with 200 ml. of fresh 2*M* ammonium hydroxide. The filtration chamber again was precoated with half a beakerful of celite, and the contents of the blendor was filtered in the chamber. The two ammonia eluates were combined, and the filter cake was discarded.

This entire process was repeated for each of the four 15-liter portions of urine. The eluates from all four chambers then were mixed. Glacial acetic acid (about 40 ml.) was added until the pH was 5.5. The acidified eluate was made up to a volume of 1,800 ml. with distilled water. Acetone (3,600 ml.) was added, and the solution was mixed and allowed to stand in the refrigerator overnight. The next morning, the clear, supernatant 66 per cent solution of acetone was removed by a siphon or an aspirator. Half a liter of 95 per cent alcohol was added to the suspended precipitate and the whole was thoroughly stirred. This mixture was placed in a filtration chamber and rapidly filtered. The precipitate, now in the form of a filter cake, was removed from the chamber, scraped from the filter paper, allowed to dry in air, and powdered. This "66 per cent acetone precipitate" (fraction A) represents in bulk the same material that is obtained from the routine processing of individual urines for clinical diagnostic purposes.

Urine of men, postmenopausal women and eunuchs was processed by this method. A total of 300 liters of urine contributed by approximately 50 normal men 20 to 60 years of age yielded about 24 Gm. of a gray-brown powder; 1,200 liters of urine contributed by

approximately 100 postmenopausal women 60 to 100 years of age yielded about 47 Gm. of a gray-tan powder; 300 liters of urine collected from about 40 eunuchs 40 to 60 years of age yielded approximately 22 Gm. of a dark-gray powder.

Assay methods

General gonadotropin assay. The method for general assay of HPG has been described in detail (3). It is performed on Sprague-Dawley female rats (bred locally) ranging in age from 21 to 23 days and in weight from 38 to 50 Gm. The HPG extract is injected in a volume of 2 ml. given subcutaneously once daily for four days. The weight of the ovaries is determined on the morning of the fifth day. The amount of HPG is determined from a standard curve that relates rat units of HPG and mean ovarian weight; 5 rat units constitute that amount of HPG which produces a mean ovarian weight of 22.5 mg. For the present study, each of the 3 preparations of HPG was administered in such amounts that the mean ovarian weight obtained fell somewhere between 22.5 and 100 mg., which is the most accurate part of the dose-response curve. In the case of normal male urine, the total amount of extract administered to each rat was the equivalent of 1.5 liters of urine. For urine from postmenopausal women and eunuchs, the total amount of extract administered represented about 0.3 liter per rat. As used in the present study, one assay for any HPG preparation was conducted on 5 rats.

Fingerprint assay. This method also has been described in detail (4). It is performed on the same type of rats used in the preceding assay. The HPG extract is injected in a volume of 2 ml. given subcutaneously once daily for four days. The total doses employed are 20, 10, 7.5, 5, 4, 3, 2 and 1 rat unit per animal, the units being previously determined by the general gonadotropin assay. Each total dose is given to each of a group of 5 rats. One fingerprint assay is conducted on 40 animals. The ovaries and uteri are weighed on the morning of the fifth day. The mean ovarian and uterine weights then are plotted as a function of dose on semilog paper. From the resulting curves, three values or parameters are calculated, namely the slope of the ovarian curve, the slope of the uterine curve, and the ratio of the threshold doses of both curves determined as intercepts at their respective control levels (ovary/uterus ratio). These three parameters are independent of the doses employed and, therefore, are independent of the differences in potency between HPG extracts. The parameters of the fingerprint curves are different for each gonadotropin thus far studied. It is anticipated that a major alteration in HPG composition, such as in its FSH/LH ratio, should be reflected by an alteration in the HPG parameters toward those of FSH or LH, as the case may be.

Ovarian-augmentation assay (for "FSH"). This assay is performed on rats purchased from Sprague-Dawley, Inc., Madison, Wisconsin. Female rats, 21 days of age and weighing 50 ± 2 Gm., are used. The extract is injected subcutaneously once daily in a volume of 2 ml. for four days. The ovaries are weighed on the morning of the fifth day. Three different total doses of the HPG extract to be assayed are administered (5 animals per dose). These three total doses are 20, 10 and 5 rat units of HPG per animal, respectively, as determined previously by the general gonadotropin assay. A second set of three doses (5 rats per dose) is mixed with human chorionic gonadotropin (Ayerst Laboratories, Inc., New York). The total doses of HPG for this second set are 10, 5 and 2.5 rat units per animal, respectively, and the total amount of HCG is 50 International Units (I.U.) per rat. In addition, 10 uninjected animals (uninjected controls) and 20 animals injected with 50 I.U. of HCG per rat (HCG controls) are included as controls. The mean ovarian weights obtained with HPG alone and with HPG plus 50 I.U. of HCG are plotted on semilog paper. A line corresponding to the mean ovarian weight for the 10 uninjected animals and another line, corresponding to the mean ovarian weight for those given

50 I.U. of HCG alone, are laid down on the y axis. Lines also are drawn on the y axis corresponding to twice the value of the mean ovarian weight induced by the HCG alone ($2y$ HCG controls) and to twice the mean ovarian weight of the uninjected animals ($2y$ uninjected controls). The following 5 values then are obtained from these curves:

1. The point on the x axis (dose) is determined where the line for $2y$ HCG controls intercepts the curve for the HPG-HCG mixture. The reciprocal of this dose is the number of ovarian-augmentation units (O.A.U.) per milligram of the HPG preparation. One unit of ovarian-augmentation activity (O.A.A.) is defined, therefore, as that amount which doubles the ovarian weight of the assay animals as compared to that of the HCG controls. This is referred to as an "FSH" unit.

2. The point at which the line for $2y$ uninjected controls intercepts the curve for HPG alone is noted on the dose scale. The reciprocal of this dose is the number of ovarian-weight units (O.W.U.) of HPG per milligram of extract. One unit of ovarian-weight activity (O.W.A.) is defined as that amount of HPG required to double the ovarian weight of the assay animals over that of the uninjected controls. This assay is similar to the general gonadotropin assay and serves as a check on it.

3. The slope of the HPG curve is determined.

4. The slope for the HPG-HCG mixture is determined.

5. The intercept of the line for $2y$ HCG controls on the curve for HPG alone is determined. This dose divided by the dose similarly obtained for the HPG-HCG curve gives the sensitivity factor (S.F.).

The main feature of this assay is the estimation of "FSH" units. The slopes and the sensitivity factor represent an additional method, reinforcing the fingerprint assay, of determining whether any great alteration in the composition of HPG is present. In general, the slopes for HPG alone are somewhat smaller than those for the HPG-HCG mixture, and the sensitivity factor is generally between 4 and 5. FSH (NIH-FSH-S1) given alone produces an extremely low slope; when augmented with HCG, however, it gives a slope equal to that of HPG-HCG mixtures, and the sensitivity factor is about 50. Therefore, it is anticipated that HPG extracts that are predominantly FSH in nature should give low slopes for the HPG alone, and the sensitivity factor should approximate 50. Each ovarian-augmentation assay is conducted on 60 animals.

Ventral-prostate assay (for "LH"). The "LH" assay is performed on rats obtained from Sprague-Dawley, Inc. These are hypophysectomized (Hormone Assay Laboratory, Chicago, Illinois) at 21 days of age; each weighs 50 ± 2 Gm. Beginning two days after operation, each animal receives a subcutaneous injection of 2 ml. of the HPG extract once daily for four days. The weight of the ventral prostate is determined on the morning of the fifth day. Three doses of the HPG extract are administered (5 animals per dose). These total doses, as determined by the general gonadotropin assay, are 10, 5 and 2.5 rat units per animal. Ten untreated animals are kept as controls. The mean weight of the ventral prostate is plotted on semilog paper. The mean weight of the ventral prostate of the uninjected controls is marked, and a line equivalent to twice this value is drawn; the point on the x axis at which this line intercepts the HPG curve is determined. The reciprocal of this dose is the number of ventral-prostate units (V.P.U.) per milligram of extract. One unit of ventral-prostate activity (V.P.A.) is defined as that amount of HPG which doubles the weight of the ventral prostate of the assay animals as compared to that of the controls. Each ventral-prostate assay is conducted on 25 animals.

Comment on assay methods. The precision for the general gonadotropin assay is about 0.10, that for the ovarian-augmentation method is approximately 0.05, and that for

the ventral-prostate assay is about 0.25. Graphic representation of both the "FSH" and "LH" assays has been given in a previous paper (5).

The 3 responses chosen for assay, namely, 100 per cent increase in ovarian weight over that in uninjected controls (O.W.A.), 100 per cent increase in ovarian weight over that in the controls injected with HCG (O.A.A.), and 100 per cent increase in ventral-prostate weight over that in uninjected controls (V.P.A.), are approximately equal in sensitivity, as the responses for V.P.A. and O.A.A. are only 1.3 times more sensitive than that for O.W.A. The close agreement in sensitivity of the 3 responses is probably fortuitous, despite the fact that the response metameter (100 per cent increase in weight over suitable controls) was the same for all.

Some of the assays summarized in the tables were conducted in pairs, one of the pair being NIH standard of FSH or LH. However, owing to the number of the animals used for each assay and the large number of assays, it was not possible to run the 3 preparations of HPG simultaneously with the appropriate standard. Consequently, each assay

TABLE 1. GENERAL ASSAYS FOR HPG: CHARACTERISTICS OF 66 PER CENT ACETONE PRECIPITATE (FRACTION A)

Source of urine	Liters	Solids (mg./L.)	General assay			Activity per liter of urine		
			No.	S.A.*	Ratio	Rat units	Mg. of HMG-20A	Ratio
Men	300	80.7	4	0.089	1	7.1	17	1
Postmenopausal women	1,200	38.5	21	0.81	9	31.2	75	4.4
Eunuchs	300	72.2	5	0.74	8	53.4	128	7.5

* Specific activity (rat units per milligram).

was calculated individually, and the results of the assay were expressed as units instead of as multiples of a standard (relative potency). Therefore, results of all such assays of the same substance performed over a period were averaged.

RESULTS

Table 1 shows the general gonadotropin assays for the 3 preparations of HPG. The specific activity of HPG from postmenopausal women and eunuchs is eight to nine times that of HPG from men. However, the yield of solid material per liter of urine from postmenopausal women is considerably less than that from normal men and eunuchs. Consequently, the gonadotropic activity per liter of urine from eunuchs is about twice that of urine from postmenopausal women. Also shown in Table 1 is the HPG content per liter of urine in terms of the unofficial standard, HMG-20A. The values for men and postmenopausal women are in general agreement with previous reports, and the value for eunuchs is confirmatory of general statements made in the literature on the high potency of urine from castrated men.

The results of the fingerprint assays are shown in Table 2. The entry for

TABLE 2. FINGERPRINT ASSAYS FOR HPG

Source of urine	No. of assays	Slopes		Ratio O./U.*
		Ovary	Uterus	
Men	6	111	254	2.0
Postmenopausal women	4	119	357	1.8
Eunchs	3	122	254	1.2

* O./U. = ovary/uterus (threshold-dose ratio).

men contains 6 fingerprint assays performed on material similar to the preparation described. These preparations were made exactly by the process described, and they differed only slightly in the total amount of solids per liter. Since the parameters of the fingerprint assays are independent of dose, all such assays performed on similar batches of male HPG are included. The values for the slopes of the ovarian and uterine curves are of the same order of magnitude for the 3 preparations of HPG regardless of the source. However, the ratio of the extrapolated threshold doses (ovary/uterus) appears to be somewhat less for the HPG of eunuchs than for the HPG of either men or postmenopausal women.

Table 3 is a summary of the augmentation ("FSH") assays. The slopes of the ovarian curves for HPG alone and for the mixture of HPG and HCG are similar, respectively, for all 3 preparations of HPG. In general, the slopes for the HPG-HCG mixture are somewhat greater than those for HPG alone. Similarly, the sensitivity factor is the same for all 3 preparations. Shown also is the mean of 3 assays for NIH-FSH-S1, an ovine FSH standard. The low slope of FSH alone is confirmatory of previous studies. When FSH is mixed with HCG, the resulting slope is of the same order of magnitude as that of HPG-HCG mixtures. The low slope of FSH alone accounts for the high sensitivity factor, which expresses numerically the extreme difference between ovine FSH and HPG in the augmentation assay. The specific activities (rat units of FSH per milligram) of the 3 preparations of HPG show the same differences in potency as were found by using general gonadotropin assays; thus, HPG (in terms of FSH) of

TABLE 3. AUGMENTATION ("FSH") ASSAYS FOR HPG

Source of urine	No. of assays	Ovarian slopes		S.F.*	Ovarian-augmentation activity				Ovarian-weight activity			
		HPG alone	HPG + HCG		R.U./mg.	Ratio	R.U./L.	Ratio	R.U./mg.	Ratio	R.U./L.	Ratio
Men	2	146	180	3.9	0.022	1	1.8	1	0.016	1	1.3	1
Postmenopasual women	2	113	179	3.8	0.21	9.5	8.1	4.5	0.16	10.0	6.2	4.8
Eunuchs	2	137	204	4.9	0.20	9.1	14.4	8.0	0.14	8.8	10.1	7.8
NIH-FSH-S1	3	52	153	51.6	9.2	—	—	—	2.9	—	—	—

* Sensitivity factor.

TABLE 4. VENTRAL-PROSTATE ("LH") ASSAYS FOR HPG

Source of urine	No. of assays	Ventral-prostate activity			
		R.U./mg.	Ratio	R.U./L.	Ratio
Men	5	0.029	1	2.3	1
Postmenopausal women	3	0.22	7.6	8.5	3.7
Eunuchs	5	0.13	4.5	9.4	4.1
NIH-LH-S1	5	24.3	—	—	—

postmenopausal women and eunuchs is about eight to nine times that of men. When calculated as units of FSH per liter of urine, the same potency ratios as obtained by the general gonadotropin assays again were found; thus, the urine of postmenopausal women is four times richer in FSH than is the urine of men, and that of eunuchs is about eight times richer than is that of men. The data regarding ovarian-weight activity in Table 3 were obtained from the dose-response lines of HPG alone and are merely confirmatory of the data in Table 1.

The ventral-prostate assays are shown in Table 4, along with the value for the NIH-LH-S1 ovine standard. The potency ratio in terms of V.P.A. for postmenopausal women and for men was the same as that obtained in terms of ovarian-weight and ovarian-augmentation activity. However, this did not hold for HPG of eunuchs. Per liter of urine, eunuchs do not excrete more LH than do postmenopausal women.

From these results (Tables 1–4), it is possible to determine the amounts of FSH and LH per liter of urine from the 3 sources, using the data obtained with ovine FSH and LH standards. These standards give valid assays in the augmentation method and in the ventral-prostate method, respectively, when paired with HPG. Therefore, the number of units of FSH per liter of urine divided by the specific activity of NIH-FSH-S1 gives the milligrams of NIH-FSH-S1 per liter of urine. A similar computation for the LH content gives the milligrams of NIH-LH-S1 per liter of urine. The ratio of these values then gives the FSH-LH ratio in the urine, which is approximately the same for men and postmenopausal women (Table 5). For eunuchs, however, this ratio is almost twice that for men and postmenopausal women. This difference is caused by the presence of

TABLE 5. "FSH" AND "LH" CONTENT PER LITER OF URINE IN TERMS OF NIH OVINE STANDARDS

Source of urine	NIH-FSH-S1		NIH-LH-S1		Ratio FSH/LH
	Mg.	Ratio	Mg.	Ratio	
Men	0.20	1	0.095	1	2.1
Postmenopausal women	0.88	4.4	0.35	3.7	2.5
Eunuchs	1.57	7.9	0.39	4.1	4.0

almost twice the amount of FSH in the urine of eunuchs as compared with that of postmenopausal women.

COMMENT

The results demonstrate a remarkable constancy in the ratios of gonadotropic potency between two sources of HPG (men and postmenopausal women), regardless of whether the potency is estimated by general gonadotropin assays or by reasonably specific FSH and LH assays. However, the urine of eunuchs is proportionately richer in FSH than LH. In addition, it is possible to estimate the amount of FSH and LH in the urine of men, postmenopausal women and eunuchs by employing the NIH standard preparations. For two reasons, it is not intended to convey the impression that the actual contents of FSH and LH are those listed in Table 5. The first reason is that the standards employed are FSH and LH from the ovine pituitary. It is likely that similar preparations made from the human pituitary would have altogether different specific activities. Were such human FSH and LH standards available and employed, the entries in Table 5 probably would be quite different with regard to both FSH and LH content and the numerical values of the FSH/LH ratio. The second reason is that the NIH standards are not pure preparations; hence, the specific activities of the standards are less than those of pure compounds. Thus, the actual content of FSH and LH per liter of urine would be less than that shown in Table 5.

The assays were not run simultaneously because of the large number of animals involved; consequently, it is not possible to calculate the usual confidence limits for many of the assays as compared with a standard. Another meaningful expression of the accuracy of the assays was calculated by determining the percentage deviation from the mean of the assays of a preparation of HPG. The assays for this study of 3 preparations of HPG were performed over a period of months, during which 6 additional preparations of HPG were tested. Thus, each of the 9 HPG preparations had at least duplicate assays, and some of the preparations were tested as many as five times. For example, one augmentation assay of an HPG preparation may have been performed in June, and the second, or duplicate, assay in September. The mean of these two assays was determined and the percentage deviation from the mean calculated. Similar deviations were calculated for all augmentation assays for the 9 preparations. The mean of these deviations was 8.3 per cent and the standard deviation was 4.4 per cent. These values describe the extraordinary accuracy of this assay. If one accepts as significant any value lying outside the range of two standard deviations, then a difference of 9 per cent (with sufficient replication) between the mean HPG content of two preparations is of acceptable

statistical accuracy. For the O.W.A., the mean deviation of all assays was 7.2 per cent and the standard deviation was 5.3 per cent. These values attest to the high accuracy of the O.W.A. test. In the case of V.P.A., the accuracy is less satisfactory, as indicated by the greater value for the index of precision. Twenty-one complete V.P.A. tests were made on 9 different preparations of HPG. The mean deviation was 7.9 per cent and the standard deviation was 8.9 per cent. Again, an allowance of two standard deviations means that a difference in "LH" potency of two different preparations of HPG would have to be greater than 18 per cent for acceptable statistical significance.

With these limits in mind, the values for FSH and LH content per liter of urine can be examined (Table 6). Little doubt exists that the FSH and LH content of the urine of postmenopausal women is different from that of

TABLE 6. RATIOS OF GONADOTROPIC ACTIVITY PER LITER OF URINE (MALE HPG = 1)

Source of urine	General assay	O.W.A.*	O.A.A.†	V.P.A.‡
Men	1	1	1	1
Postmenopausal women	4.4	4.8	4.5	3.7
Eunuchs	7.5	7.8	8.0	4.1

* Ovarian-weight activity.
† Ovarian-augmentation activity.
‡ Ventral-prostate activity.

men, the differences being 450 and 370 per cent, respectively. The FSH content of HPG from eunuchs is also greater than that from postmenopausal women (77 per cent). However, the LH content of HPG from eunuchs is not greater, the difference being 11 per cent, which is well within the range of variability of duplicate assays as expressed by the value of 18 per cent for two standard deviations.

It is of some significance that the potency of postmenopausal HPG is four to five times that of male HPG when measured by a general gonadotropin assay or by assays for LH or FSH. This can be interpreted in two ways, namely that HPG is a single molecule with both FSH and LH activities, something like the gonadotropin of pregnant-mare serum (PMS), or that HPG is a mixture of FSH and LH and that postmenopausal HPG contains four to five times more of both FSH and LH than does male HPG. That the latter of these interpretations is the more likely is indicated by the comparison of HPG from eunuchs with that from men or postmenopausal women. This comparison is seen best in Table 6, which summarizes the ratios of activities per liter of urine for the 3 preparations of HPG. For the general gonadotropin and FSH assays, the ratios parallel one another. However, for the LH assay, the parallelism fails with HPG from eunuchs.

This lack of parallelism is suggestive but not absolute evidence that HPG is a mixture of two gonadotropins.

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IODINE METABOLISM IN SURVIVING THYROID SLICES FROM PATIENTS WITH EXOPHTHALMIC GOITER*

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ABSTRACT

Surviving slices of hyperplastic thyroid tissue from 18 patients with exophthalmic goiter prepared for operation with strong iodine solution (Lugol's solution), and of normal thyroid tissue from 4 euthyroid patients were incubated with a solution of Krebs-Henseleit bicarbonate medium and radioiodine by a gravity-flow method. Iodine metabolism in the hyperplastic tissue differed from that of normal tissue in three respects: 1) the pattern of I^{131} uptake in hyperplastic tissue was curvilinear with respect to time, whereas that of normal tissue was rectilinear; 2) iodine transport was greater in hyperplastic tissue than in normal tissue; and 3) the mean proportion of I^{131} bound to protein was less in hyperplastic tissue than in normal tissue. In both hyperplastic and normal tissue, binding of I^{131} to protein was blocked by 10^{-3} *M* methimazole (Tapazole). In the presence of methimazole, the accumulated radioiodine was discharged, but not completely, by 0.2 *M* thiocyanate. Thyroid-to-medium ratios of less than unity resulted. Although certain concentrations of inorganic iodide inhibited both I^{131} trapping and protein binding, organic iodide compounds (thyroxine, triiodothyronine, diiodotyrosine and thyroglobulin) had no effect on either the trapping or the binding function of exophthalmic goiter tissue.

MORTON and Chaikoff (1, 2) and Schachner, Franklin and Chaikoff (3) studied iodine metabolism in surviving animal thyroid slices by adding radioiodine to a fixed volume of incubating medium. They and others noted that stable iodine added to the medium interfered with the accumulation of radioiodine in thyroid slices (4–7). Molnar and associates (8) found that appreciable quantities of I^{127} were leached from thyroid slices into the incubating medium; hence, the specific activity of iodine in the medium was lowered and this interfered with the accumulation of I^{131} by the slice. A method that provided a medium of constant composition flowing by gravity at a constant rate over thyroid slices was devised to obviate this difficulty (9). This method of gravity-flow incubation was

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utilized by Schumacher, Keating and Albert (10) in a study of iodine metabolism in several thyroid disorders. The purpose of this paper is to report similar studies on thyroid slices obtained from patients with exophthalmic goiters.

MATERIALS AND METHODS

Surviving thyroid slices from 18 patients with exophthalmic goiter and from 4 euthyroid subjects were studied. All patients with exophthalmic goiter had been prepared for operation with strong iodine solution (Lugol's solution) for two to one hundred and twenty days. In one instance, tissue was obtained before and after such treatment. Patients with nodular goiters were not included. Thyroid tissue was obtained and prepared by the method described by Molnar and associates (8).

The technic of gravity-flow incubation, described by Molnar and associates (9), was slightly modified for the present study. The incubating medium, Krebs-Henseleit bicarbonate, contained 4 microcuries of I^{131} per 100 ml. Uniform temperature (38° C.) was maintained by two thermostatically controlled water baths, one containing the reservoirs and the other the tissue chamber vessels and connecting plastic tubing. Radioactivity was measured with a sodium iodide (thallium-activated) scintillation counter connected to a model 1070-A Multiscaler (Atomic Instrument Co.).

Four to six tissue chamber vessels, each containing two thyroid slices, were used depending upon the plan of the experiment. This permitted one, and more frequently two, of the chambers to act as a control for each experiment. The rate of flow was 0.5 ml. per minute and the period of incubation was one hundred and eighty minutes. Organically bound radioactive iodine in the incubated tissue slice was determined by precipitation with trichloracetic acid.

RESULTS

Iodine metabolism in slices of thyroid tissue from patients with exophthalmic goiter differed from that of normal thyroid tissue in three respects: 1) The pattern of I^{131} uptake in hyperplastic tissue was curvilinear with respect to time, whereas that of normal tissue was rectilinear (Fig. 1). 2) The rate of I^{131} uptake was greater in hyperplastic tissue than in normal tissue. The mean thyroid-to-medium (T/M) ratios were 16.4 $\pm$2.0 (S.D.) and 5.0 $\pm$0.9 respectively for hyperplastic and normal tissue when protein binding was blocked by 10^{-3} *M* methimazole (Tapazole), and 17.2 $\pm$1.4 and 9.7 $\pm$2.5 when protein binding was permitted. 3) The mean proportion of I^{131} bound to protein in hyperplastic tissue was 35.8 $\pm$3.1 per cent as compared to 72.6 $\pm$4.8 per cent in histologically normal tissue.

Effect of methimazole on protein binding. Eleven tissue slices from 8 patients with exophthalmic goiter were incubated in the presence of 10^{-3} *M* methimazole. Protein binding was effectively blocked by methimazole, the mean trichloracetic acid precipitable I^{131} (TPI^{131}) being 1.4 $\pm$0.1 per cent in hyperplastic tissue and 1.8 $\pm$0.2 per cent in normal tissue.

Effect of thiocyanate. The effect of thiocyanate was similar in normal and hyperplastic tissue. After tissue slices were incubated for one hundred and

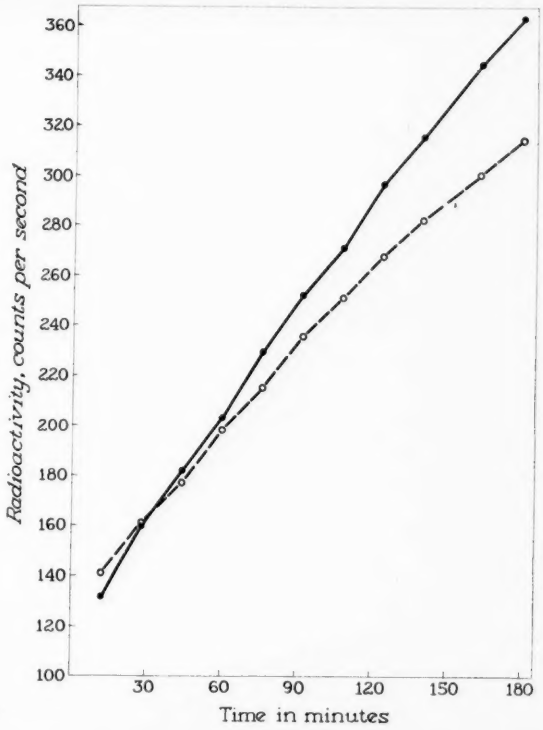

FIG. 1. Curvilinear pattern of I^{131} uptake of hyperplastic tissue when gross uncorrected counts of accumulating radioactivity (as counts per second) were plotted against time. No correction was made for the weight of the slices. The slice represented by dots weighed 140 mg.; by circles, 130.5 mg.

eighty minutes with medium containing 10^{-3} *M* methimazole, similar medium containing 0.2 *M* thiocyanate was introduced. Despite continued availability of I^{131}, no further uptake occurred. Thiocyanate discharged most, but not all, of the accumulated radioiodine. The mean T/M ratio after thiocyanate discharge was 0.83 for normal tissue and 0.95 for hyperplastic tissue.

Effect of inorganic iodide. In two experiments, slices of hyperplastic tissue were incubated with standard medium and with media to which 10 and 100 μg. per 100 ml. of stable iodide (I^{127}), as sodium iodide, had been added (Table 1). The mean T/M ratio was reduced by 45 per cent and 34 per cent with the medium containing 100 μg. of I^{127} but was unchanged with that containing 10 μg. The mean proportion of I^{131} which was bound to protein (TPI^{131}) was reduced by 47 per cent and 60 per cent with 10 μg. of

TABLE 1. EFFECT OF I^{127} ON THYROID-TO-MEDIUM RATIO AND ON PROTEIN BINDING OF HYPERPLASTIC TISSUE

Thyroid-to-medium ratio			Per cent of I^{131} protein bound		
Control*	I^{127} added to medium (per 100 ml.)		Control*	I^{127} added to medium (per 100 ml.)	
	10 μg.	100 μg.		10 μg.	100 μg.
17.6	20.1	9.5	24.4	13.2	6.2
21.2	20.1	11.9	33.8	12.2	5.6
26.6	29.1	21.1	14.0	4.7	2.5
36.9	27.7	20.4	11.1	5.2	3.1

* Medium contained less than 1.0 μg. of I^{127} per 100 ml.

I^{127} per 100 ml. and by 75 per cent and 78 per cent with 100 μg. of I^{127} per 100 ml.

Effect of treatment with strong iodine solution. In one instance, four thyroid slices were studied before and after the patient was treated with Lugol's solution (10 drops three times daily for eight days). This treatment reduced the T/M ratio for I^{131} from 28.2 ± 2.0 to 20.5 ± 0.9, but was more effective in reducing the proportion of I^{131} which was bound to protein (TPI^{131} lowered from 29.1 ± 2.2 per cent to 9.0 ± 1.3 per cent).

Effect of organic compounds. Table 2 records the results when L-diiodo-

TABLE 2. EFFECT OF ORGANIC COMPOUNDS ON THYROID-TO-MEDIUM RATIO AND ON PROTEIN BINDING OF HYPERPLASTIC TISSUE

Thyroid-to-medium ratio		Per cent of I^{131} protein bound	
Control*	μg. per 100 ml.	Control*	μg. per 100 ml.
	100 μg. L-diiodotyrosine		100 μg. L-diiodotyrosine
17.9	12.7	52.8	46.0
13.4	15.8	49.2	56.5
	20 μg. L-triiodothyronine		20 μg. L-triiodothyronine
15.2	15.2	43.9	34.4
18.1	13.7	44.3	33.9
	60 μg. L-triiodothyronine		60 μg. L-triiodothyronine
9.8	9.1	71.9	70.4
11.9	10.2	62.9	60.3
	60 μg. L-thyroxine		60 μg. L-thyroxine
24.8	25.7	28.6	29.6
21.7	24.2	21.7	25.4
	100 μg. I^{127} as thyroglobulin		100 μg. I^{127} as thyroglobulin
6.5	8.9	30.2	41.7
10.4	7.0	41.2	33.0

* Medium contained less than 1.0 μg. of I^{127} per 100 ml.

tyrosine, 3,5,3′-L-triiodothyronine, L-thyroxine, or thyroglobulin was added to the medium. The T/M ratio was not significantly changed by any of the compounds tested. In one instance, the TPI^{131} was reduced when 20 μg. of L-triiodothyronine per 100 ml. was added to the medium, but no change was noted in another instance when 60 μg. of L-triiodothyronine per 100 ml. was added.

COMMENT

The uptake of iodine in normal tissue was linear with respect to time, as previously reported by Molnar and co-workers (9). However, except in one instance, the uptake pattern of hyperplastic tissue, including that obtained before treatment with Lugol's solution, was slightly curvilinear. Loss of protein-bound I^{131} from tissue slices into the incubating medium did not explain the curvilinearity, since similar uptake patterns were obtained when protein binding of I^{131} was blocked by methimazole. A curvilinear uptake pattern was observed in slices of normal tissue when protein binding was blocked with methimazole. The similarity of these uptake patterns suggests that hyperplastic tissue slices, incubated with medium containing less than 1 μg. per 100 ml. of inorganic iodide, were partially blocked.

That the thyroid in exophthalmic goiter has an increased avidity for iodine has been demonstrated repeatedly. The iodine-accumulating capacity of hyperplastic tissue, as reflected in the T/M ratio, was similar whether protein binding was permitted or blocked. Ingbar (11) observed that the mean *in vivo* thyroid to plasma ratio in untreated Graves' disease was about three times that in normal subjects. Although relatively lower gradients were observed with gravity-flow incubation, the iodide trap in exophthalmic goiter tissue *in vitro* was similarly found to be about three times that of normal tissue.

The mean proportion of I^{131} bound to protein in hyperplastic tissue was about half that in normal tissue. This is in agreement with the findings of Molnar and co-workers (9). Braasch and associates (12) showed that after treatment with Lugol's solution only 68 per cent of the iodine in exophthalmic goiter was protein bound as compared to 95 per cent before treatment. By this *in vitro* method, it was shown that treatment with strong iodine solution for eight days reduced the TPI^{131} from 29.1 ± 2.2 per cent to 9.0 ± 1.3 per cent.

Once thiocyanate was introduced, despite continued availability of I^{131}, no further uptake occurred. This is in agreement with the *in vitro* findings of Franklin, Chaikoff and Lerner (13) and the *in vivo* studies of Wolff and associates (14) and VanderLaan and Bissell (15). Thiocyanate promptly discharged most but not all of the accumulated radioiodine in a pattern similar to that reported by Berson and Yalow (16). A greater proportion of

the I^{131} was discharged when protein binding was blocked with methimazole. In this study, T/M ratios of less than unity were obtained both in normal and in hyperplastic tissue.

When 10 μg. of stable iodide was added to 100 ml. of medium, a significant depression of T/M was not observed, but the TPI^{131} was reduced by approximately 50 per cent. Protein binding was almost completely inhibited by 100 μg. of I^{127} per 100 ml. (TPI^{131}, 6 per cent and 3 per cent). Similar results were obtained in tissue studied before and after treatment with Lugol's solution. Although iodide trapping capacity was somewhat reduced, greatly increased inhibition of protein binding was observed.

Since Plummer (17) introduced iodine therapy for exophthalmic goiter, the mechanism whereby excess iodide exerts its ameliorating effect has been studied extensively. Most studies have demonstrated inhibition of protein binding by iodide. Wolff and Chaikoff (18) postulated that the level of plasma iodide was part of a homeostatic mechanism regulating hormone synthesis. Halmi and associates (19, 20) proposed a so-called intrinsic thyroid principle which inhibits the iodide-concentrating mechanism. They stated that this principle might be "one of the several organic iodide-containing compounds in the thyroid whose concentration under most circumstances is assumed to parallel that of the total thyroidal iodine and which could be the thyroid hormone proper." Molnar and associates (9) found depression of I^{131} uptake in normal tissue with L-triiodothyronine, L-thyroxine and thyroglobulin. In this study, L-diiodotyrosine, L-triiodothyronine, L-thyroxine, or thyroglobulin did not produce significant changes in the T/M ratio or in protein binding of tissue from exophthalmic goiter.

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Letters to the Editor

METHOD OF PURIFICATION OF HUMAN PITUITARY GONADOTROPIN FOR CLINICAL ASSAYS*

To the Editor:

For routine clinical purposes, most laboratories process urine for the assay of human pituitary gonadotropin (HPG) by a kaolin-acetone (K.A.) method. The K.A. method of Albert (1) yields about 70 mg. of solids per liter of urine from normal men. The HPG concentrate obtained by this technic is not toxic when the assay is performed on rats. However, it has been reported (2) that when mice are used as the assay animals, 12 per cent of the routine tests cannot be completed because of toxicity. The toxicity of the concentrate may be associated with the relatively large amount of solids administered to the mice. Another difficulty, also presumably related to the high solid content of the K.A. concentrate, is that some new and sensitive biologic methods for the detection and study of gonadotropins cannot be employed because it is not possible to dissolve the concentrate in the small volumes required for intravenous injection. Therefore, it appears that a considerable reduction in the total solids of the K.A. concentrate without great loss in HPG content would be of value, 1) in reducing toxicity when mice are used as the assay animals, 2) in making it possible to determine HPG by new biologic tests now available, and 3) in furnishing basic procedures whereby HPG eventually may be measured by other than biologic methods.

We recently have shown that the HPG of urine from normal men can be purified 50-fold with a loss of approximately 30 per cent of the total HPG activity (3). This chemical method results in the reduction of the solid content of the K.A. concentrate from about 70 mg. per liter of urine, as routinely obtained, to approximately 1 mg. per liter. The application of this method to small quantities of urine (48-hour specimens such as are used in routine diagnostic work) is the subject of this report.

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METHOD

1. Adjust 3 liters of male urine (representing approximately a 48-hour sample) to pH 4.5 with glacial acetic acid. Add 9 Gm. of celite, stir thoroughly and filter under positive pressure. Discard the celite. To the filtered urine, add 20 Gm. of kaolin, stir and refilter. Discard the urine. Wash the kaolin cake with 2 liters of 1 per cent solution of sodium chloride at pH 4.5, and discard the saline wash.

2. Elute the HPG with 2 *M* ammonium hydroxide as previously described (1). Acidify the eluate to pH 5.5 with glacial acetic acid, mix with 2 volumes of acetone and collect the precipitated HPG as previously described (1). Transfer the HPG precipitate to a 40-ml. centrifuge tube with the aid of washings with 66 per cent acetone. Centrifuge and discard the supernatant 66 per cent acetone.

3. Extract the moist precipitate twice with 7 ml. of 10 per cent solution of ammonium acetate in 70 per cent ethyl alcohol. Combine the two extracts and discard the insoluble material. To the combined extract, add 2 volumes of absolute alcohol saturated with ammonium acetate and place in a refrigerator overnight.

4. The next morning, centrifuge and discard the supernatant alcohol. Wash the precipitate by emulsifying it in 30 ml. of 90 per cent ethyl alcohol. Centrifuge, and discard the supernatant alcohol.

5. Dissolve the precipitate in 10 ml. of 0.05 *M* phosphate buffer. Add 200 mg. of diethylaminoethyl cellulose (DEAE-C), stir and centrifuge. Save the supernatant fluid. Extract the DEAE-C with another 10 ml. of 0.05 *M* phosphate buffer and centrifuge. Combine the extracts and discard the DEAE-C. Filter the combined extract through rough filter paper previously moistened with 0.05 *M* phosphate buffer in order to remove any floating strands of DEAE-C.

6. To the filtrate add $\frac{1}{10}$ volume of 10 per cent sodium chloride, 1 ml. of 10 per cent tannic acid, and 10 per cent acetic acid drop by drop to a pH of 5.0. Let stand for one hour at room temperature. Centrifuge, and discard the supernatant fluid. Wash the tannate by emulsifying it in 30 ml. of 90 per cent ethyl alcohol containing 1 drop of 10 per cent sodium chloride to remove traces of excess tannic acid. Centrifuge, and discard the alcohol wash. Dissolve the precipitate in 1 ml. of borate buffer at pH 8.0 (if any insoluble material is present, add 1 *N* sodium hydroxide drop by drop just until a clear solution results) and then dilute with borate buffer to the desired volume for assay. Usually, the precipitate from 3 liters of urine will dissolve in 0.5 ml. of borate buffer—a 6,000-fold reduction in volume as compared with the original urine.

EFFICACY OF METHOD

The efficacy of this method is shown in Table 1. A 12-liter batch of urine from normal men was divided into four aliquots of 3 liters each. The first two aliquots were processed as controls by the routine procedure previously described (1). The third and fourth aliquots were processed as described here in the six steps. This experiment was repeated 10 times. The mean loss in HPG potency was 25 per cent. The solid content of the experimental aliquots varied between 1 and 2 mg. per liter of urine.

COMMENT

This method was evolved after about 500 experiments conducted in the manner outlined in the table, but with various modifications in order to

determine the optimal conditions under which small samples of urine could be handled. The following comments pertain to the reasons for the introduction of the six steps described, and to the limits within which they may operate.

The preliminary filtration of urine with celite was undertaken to reduce the solid content obtained in the K.A. concentrate and thus effect reduction in the volume of the reagents used in subsequent steps. The amount of celite (3 Gm.) for each liter of urine is optimal. No loss in HPG occurs and the solid content is reduced to approximately half (range from 15 to 60 per cent) of the control values, or about 30 mg. of solids per liter of urine.

TABLE 1. RECOVERY OF HPG (R.U./1.5 LITERS) DURING PURIFICATION PROCEDURE ON MALE URINE

No.	Control		Experimental	
	1	2	3	4
1	29.0	27.2	15.0	14.0
2	25.8	25.8	26.1	19.0
3	33.8	15.6	24.2	23.0
4	26.8	27.2	23.0	22.5
5	16.2	25.8	22.5	13.7
6	24.2	16.2	9.0	12.0
7	25.1	16.2	18.2	16.9
8	35.0	26.1	27.2	18.2
9	38.5	19.3	21.1	16.6
10	24.2	24.2	13.4	18.5
Mean	25.06		18.70	

Mean loss = 25 per cent

Greater amounts of celite induce loss of HPG, whereas lesser amounts adsorb smaller quantities of impurity.

The extraction of the 66 per cent acetone precipitate with 10 per cent ammonium acetate in 70 per cent alcohol represents the only step in which appreciable loss of HPG occurs. This loss varies between 10 and 40 per cent, but it achieves a purification of about 10-fold. After precipitation of the HPG with 90 per cent ethyl alcohol containing about 10 per cent ammonium acetate, the precipitate is washed with 90 per cent ethyl alcohol to remove adhering ammonium acetate which would interfere with the next step. The solid content of the extract at this point varies between 4 and 6 mg. per liter.

The use of 0.05 *M* phosphate buffer in the next step, when additional impurity is adsorbed by DEAE-C, is critical. The use of a more dilute buffer leads to adsorption of HPG to the cellulose; a more concentrated buffer decreases the amount of impurity removed by the cellulose. It is also critical that the ratio of solid content to DEAE-C be of the order of magni-

tude of 1:10. Since about 15 mg. of solids are present from 3 liters of male urine at this point, an amount slightly in excess of this ratio, namely 200 mg. of DEAE-C, was used routinely. No loss in HPG potency occurs at this stage. The use of tannic acid (well known to precipitate HPG quantitatively) was introduced to reduce the volume of the extract. The conditions for the preparation of the tannate are critical.

Specimens of urine (24-hour or 48-hour collections) from young women and postmenopausal women have not been studied in this fashion. However, the procedure for the purification of male urine in bulk (3) works just as effectively on large quantities of postmenopausal urine, leading to the preparation of HPG with high specific activity. Therefore, the method described should also be effective for specimens of urine from young and old women.

SUMMARY

A method for the concentration of human pituitary gonadotropin from 48-hour samples of urine of normal men is described wherein the content of solids has been reduced from about 70 mg. per liter to about 1 mg. per liter, with a net recovery of about 75 per cent of the gonadotropic potency originally present.

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UNUSUAL STEROID PATTERN IN CONGENITAL ADRENAL HYPERPLASIA: DEFICIENCY OF 3β-HYDROXY DEHYDROGENASE

To the Editor:

A hitherto undescribed urinary steroid pattern for the salt-losing adrenogenital syndrome has been observed in 3 infants with this disorder. The C-21 methyl corticosteroids with 3α-hydroxy saturated ring structure most commonly associated with this disorder (1) were absent. A group of 3-β-hydroxy-Δ^5 steroids predominated. These findings suggest yet another enzymatic defect as a cause of the syndrome, presumably involving 3β-hydroxy steroid dehydrogenase. This form of the disease is serious, since all the patients died. A preliminary communication is submitted so that early attention may be given to this important clinical problem, thus avoiding possible diagnostic confusion arising from the absence of urinary pregnanetriol, pregnanetriolone and 17-hydroxypregnanolone (Nos. 3, 4 and 5 in Table 1).

The steroids were hydrolyzed by incubating the urine with mammalian liver β-glucuronidase, and were exhaustively extracted with methylene chloride and ethyl acetate. The urine was then submitted to solvolysis in tetrahydrofuran with perchloric acid according to principles set forth by Burstein and Lieberman (2). The separate extracts were fractionated with digitonin prior to chromatography. Further phases of the methods were according to published details, annotated for each compound in Table 1. Most steroids were identified by mobilities in chromatographic systems, sulfuric acid chromogens, melting points and mixed melting points (both free and acetates), and when indicated, by infrared analysis. Substances present in amounts less than 1.0 mg. per day were characterized by the first two criteria alone. Table 1 shows the findings in the 3 cases studied (A, B, C) and in 1 "classic" case (D) investigated earlier. Case D is representative of 9 infants with the salt-losing form of congenital adrenal hyperplasia examined in this department.

The predominant steroids isolated (Nos. 8, 9, 10 and 11) were present in the extracts following solvolysis and in the digitonin precipitable fraction. In addition, a more polar ketonic compound was present in all extracts; it reacted weakly in the Zimmermann test but reduced triphenyl tetrazolium. This may represent Δ^5-androstene-3β,7α,16α-triol-17-one, but definitive characterization has not been possible. Finally, a highly polar nonketonic steroid, which was precipitable with digitonin and yielded formaldehyde on periodate oxidation, was isolated. As yet, it has not been identified. This suggests the possibility of 21-hydroxylation of the 3β-OH series.

Insufficient urine was available in Patient A for complete analysis.

Patients A and B were female pseudohermaphrodites with the characteristic masculinization of the external genitalia, and each was known to have a similarly affected sibling. Patient C was a proven male with hypospadias. Autopsy was performed on only one patient and on the sibling of another. The postmortem findings were consistent with the diagnosis of congenital adrenal hyperplasia.

Upon administration of hydrocortisone, the total neutral urinary 17-

TABLE 1. STEROIDS IN URINE (MG. PER 24 HRS.)

Steroid	Subjects of this study			"Classic" case	References for method used
	A Female, age 1 wk.	B Female, age 2 wks.	C Male, age 8 wks.	D Male, age 2 wks.	
1. Total 17-ketosteroids	6.2	7.0	10.2	3.9	(3)
2. Allen chromogens	8.0	4.5	7.5	0.2	(4)
3. Pregnane-3α,17α,20α-triol	0	0	0	1.5*	(1)
4. Pregnane-3α,17α-diol-20-one	0	0	0	1.2*	(1)
5. Pregnane-3α,17α,20α-triol-11-one	0	0	0	3.6*	(1)
6. Pregnane-3α,17α,21-triol-11,20-dione (Tet. E)	0	0	0	0.02	(5)
7. Pregnane-3α,17α,21-triol-20-one (Tet. S)	0	0	0	0	(5)
8. Δ^5-Pregnene-3β,17α,20α-triol	4.0	3.8*	4.9*	0.1	(5)
9. Δ^5-Pregnene-3β,20α-diol	—	2.0	2.2	0	(7)
10. Δ^5-Pregnene-3β,17α-diol-20-one	—	2.1	3.9*	0	(6)
11. Δ^5-Androstene-3β-01-17-one	—	3.1	4.5	0.2	(6)
12. Δ^5-Androstene-3β,16α-diol-17-one	—	2.0*	2.9*	0	(6)
13. Androsterone	—	0.8	1.0	1.0	(7)
14. Etiocholanolone	—	—	0.2	0.6	(7)

* Infrared identification.

ketosteroids fell to normal levels for the ages of the subjects. In Patients B and C (studied in greater detail), the Allen chromogens also diminished and the unusual steroidal metabolites listed in the table disappeared. In all 3 patients the synthesis of hydrocortisone was inadequate, as reflected by the absence of tetrahydro-E. Highly polar metabolites of hydrocortisone were not present. The urine of the single normal male infant examined contained smaller quantities of the compounds (Nos. 8, 9, 10, 11) characteristic of this form of the adrenogenital syndrome.

Although these patients are reminiscent of the one reported by Bradbury and Slate (8), the findings in the latter case do not appear to be pertinent. Their patient was a female without any malformation of the genitalia and hence may not have represented congenital adrenal hyperplasia. The single unusual finding reported was a preponderance of dehydroisoandrosterone.

There was no clinical evidence of lack of hydrocortisone, and there was no information concerning the excretion of the other steroids listed here.

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Acknowledgment

The author is most indebted to Dr. and Mrs. Thomas Gallagher for the infrared identifications. Dr. David Fukushima and Dr. Seymour Bernstein kindly supplied certain steroids. Dr. Luigi Luzzati and Dr. Paul Gyorgy provided 2 of the cases.

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PROGESTIN IN THE BLOOD OF NEWBORN INFANTS*

TO THE EDITOR:

The purpose of this investigation was to search for progestin in the peripheral blood of male and female infants during the first four days after birth. Information as to the possible presence of the hormone in such blood during this period seems not to have been published previously.

* This research was supported by research grants RG-2342 and A-2504(C2) from the National Institutes of Health, Public Health Service.

MATERIALS AND METHODS

Blood samples were collected from the peripheral tissues of 16 female and 14 male infants at periods ranging from seven to ninety-nine hours after normal birth at full term (Fig. 1). Only one sample was taken from each donor. The blood was placed in a centrifuge tube which in some cases contained 0.05 ml. of 20 per cent sodium citrate solution as an anticoagulant. After brief centrifugation of the specimen, a measured volume of plasma or serum was removed. With the exception of 5 specimens, all samples were kept continuously refrigerated or frozen until bio-assay. (Freezing of the 5 specimens was interrupted during shipment.) All specimens were prepared and were bio-assayed in CHI mice for protein-"free" (*i.e.*, acetone- and ether-soluble) progestin by the method of Hooker and Forbes (1–3). Some specimens were also bio-assayed for protein-"bound" progestin. Because the plasma (not including anticoagulant) and serum specimens were relatively small (from 0.03 to 0.40 ml. in volume), it was assumed that quantitative errors might be very large; results are therefore reported only on the basis of progestin being either detectable or not detectable. Further, since the lower limit of

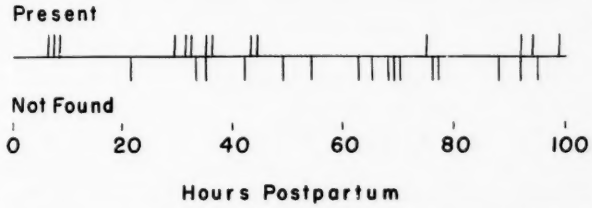

FIG. 1. Presence or absence of progestin in peripheral blood samples.

sensitivity for progesterone when bio-assayed by this method in the CHI mouse is about 0.25 μg. per ml. of vehicle, it is possible that progestin was present, but only at less than detectable levels, in some of the smaller samples recorded as containing no progestin.

RESULTS

"Free" progestin was detected in the peripheral blood of 10 of the 14 infants bled before the forty-fifth hour post partum (Fig. 1), but it was not detected in the blood of 12 of the 16 infants bled from the forty-ninth to the ninety-ninth hour. Presence or apparent absence of progestin was not correlated with the sex of the donor. The hormone was found in all 5 of the specimens for which refrigeration had been interrupted.

"Bound" progestin was found in 7 of the 17 specimens in which the "bound" fraction was assayed. With one exception (ninety-two hours), "bound" progestin was detected only in those samples which also contained "free" hormone.

DISCUSSION

The progestin found in the blood of the newborn infant presumably: *a*) was secreted prior to birth by the mother, the placenta or the fetus, or by some combination of these sources, and had not disappeared from the

infant's blood by the time the sample was drawn, or *b*) the progestin was produced by the infant during the postnatal period, or *c*) the hormone was released during both the prenatal and postnatal periods. The rate of clearance of exogenous progesterone is very rapid in adult human beings (4–6); all or nearly all of injected progesterone, as represented in the blood by this hormone or its active metabolites, disappears from the blood in two hours or less (7–10). No information has been found as to the rate of clearance of progestin from the blood of the infant.

Hoffmann and Uhde (11) assumed that the human fetus inactivates little progesterone. They report finding the hormone in the urine of the infant for as long as ten days post partum, and suggest that progesterone is secreted by the adrenal cortex of the newborn child. Philipp (12) also stated that he found progesterone in the urine for the first three days after birth, but believed the hormone had come from the placenta. These investigators used bio-assays. Since progesterone cannot be detected in the adrenal of the newborn infant by physicochemical tests (13, 14), a lack of specificity in the biologic tests is indicated.

Though the present study sheds no light on the source of the progestin found by bio-assay in the blood of the newborn infant, the fact that the hormone is usually present for the first two days after birth raises some challenging questions.

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THYROID FUNCTION IN A CASE OF PHENYLKETONURIA

TO THE EDITOR:

In phenylketonuric oligophrenia the basic defect is lack of, or incomplete, hydroxylation of phenylalanine to tyrosine. Thus it would seem possible that the thyroidal tyrosine pool would diminish to such an extent as to influence the kinetics of iodine metabolism. Earlier studies have been confined merely to determination of the basal metabolic rate, with variable results. Recently Coppen (1) reported normal values for the serum protein-bound iodine (PBI) level in 14 cases of phenylketonuria. The present authors recently had opportunity to study the iodine metabolism in such a patient more closely and it seemed of interest to report the observations.

MATERIALS AND METHODS

The patient was a girl, aged 14 years, with the mental status of an idiot. The full case record, including additional studies, will be reported elsewhere. She was given approximately 300 μc. of radioactive iodine (I^{131}) (The Radiochemical Centre, U.K.A.E.A., Amersham, England) intravenously. Blood samples were drawn on subsequent days and analyzed for total and protein-bound radioactivity. It was not possible to collect the urine, owing to the patient's mental status. The results of direct measurement of thyroidal I^{131} uptake were also inaccurate because the patient was restless. The thyroid appeared to be normal on palpation.

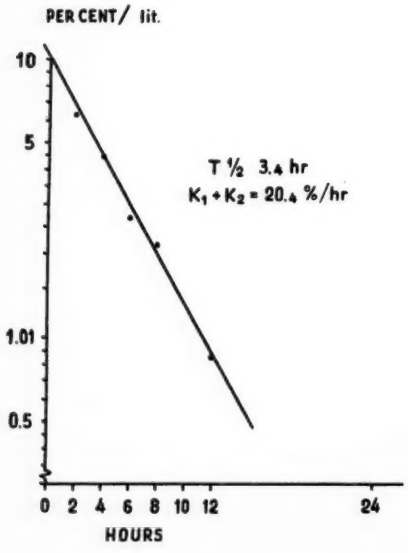

FIG. 1. The disappearance of radioactive iodine given intravenously.

RESULTS

The results are depicted in Figures 1 to 3. Iodide was rapidly removed from the plasma, a phenomenon commonly observed in people living in the endemic goiter area of Finland where the patient also lived. The early disappearance curve for I^{131} (Fig. 1) showed a half-time of about 3.4 hours, indicating a total disposal rate of 20.4 per cent per hour (2). The total disposal rate involves two disappearance rates, the renal (k_2) and the thyroidal rate (k_1) (2). Assuming a value of 9 per cent per hour for k_2, then k_1 would be 11 per cent per hour, which is higher than normal (3) but common in endemic goiter in Finland (4). K_2 was found to be 7 per cent per hour by Stanbury and co-workers (3), and 9.5 per cent per hour by the authors (4) (endemic goiter in Finland).

The 24-hour conversion ratio was 22 per cent. The level of serum PBI^{131}, as determined after precipitation with trichloracetic acid, was within the normal range after forty-eight and seventy-two hours (Fig. 2). Later it rose to approximately 0.5 per cent per liter, which is fairly high but not definitely abnormal. The serum PBI level was 5.6 and 5.0 μg. per 100 ml. on two occasions. The thyroxine-binding capacity was determined according to the method of Dowling, Freinkel and Ingbar (5) and was in the normal range, *i.e.*, binding in albumin and TBP equal at 60 μg. per 100 ml.

About 90 per cent of the radioactive iodine of plasma was extractable with butanol. Radiochromatography revealed the presence of thyroxine and a trace of iodide (Fig. 3).

The 24-hour thyroidal uptake of I^{131} was 29 per cent. The maximal uptake calculated according to the disposal rates (3) was 56 per cent.

On the ninth day, a dose of 10 USP units of thyrotropin (Actyron®, Ferring, Sweden), divided into two doses of 5 units each, was administered

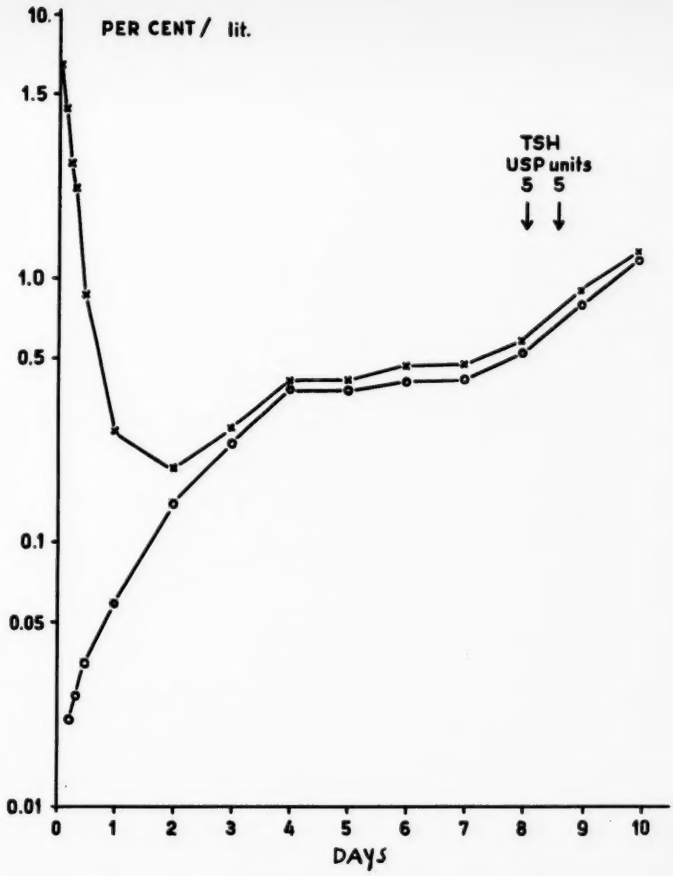

FIG. 2. Plasma total (x——x) and protein-bound (○——○) radioactive iodine levels after intravenous administration of I^{131}, and the effect of TSH.

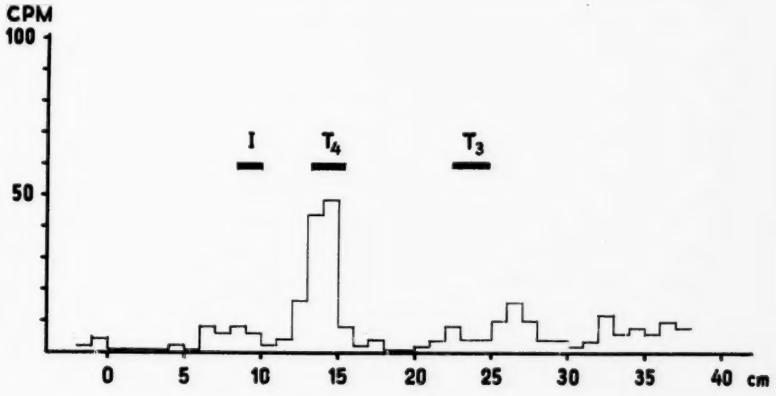

FIG. 3. Radiochromatography of serum.

intramuscularly. The response was normal; the serum PBI level rose to 9 μg. per 100 ml. on the second day after stimulation and the PBI^{131} level rose to 1.1 per cent per liter (Fig. 2).

Intrathyroidal iodine metabolism was apparently normal in this case of phenylketonuria. The findings are not compatible with a "low thyroid reserve syndrome" (6). The thyroidal tyrosine pool was most probably filled by nutritional tyrosine, although there may have been other pathways of metabolism.

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The Endocrine Society

Recipients of the 1961 Awards

THE FRED CONRAD KOCH AWARD

Lawson Wilkins:

In affection and esteem, we hail you and herewith confer upon you the highest honor we have in our power to bestow: The Koch Medal of The Endocrine Society.

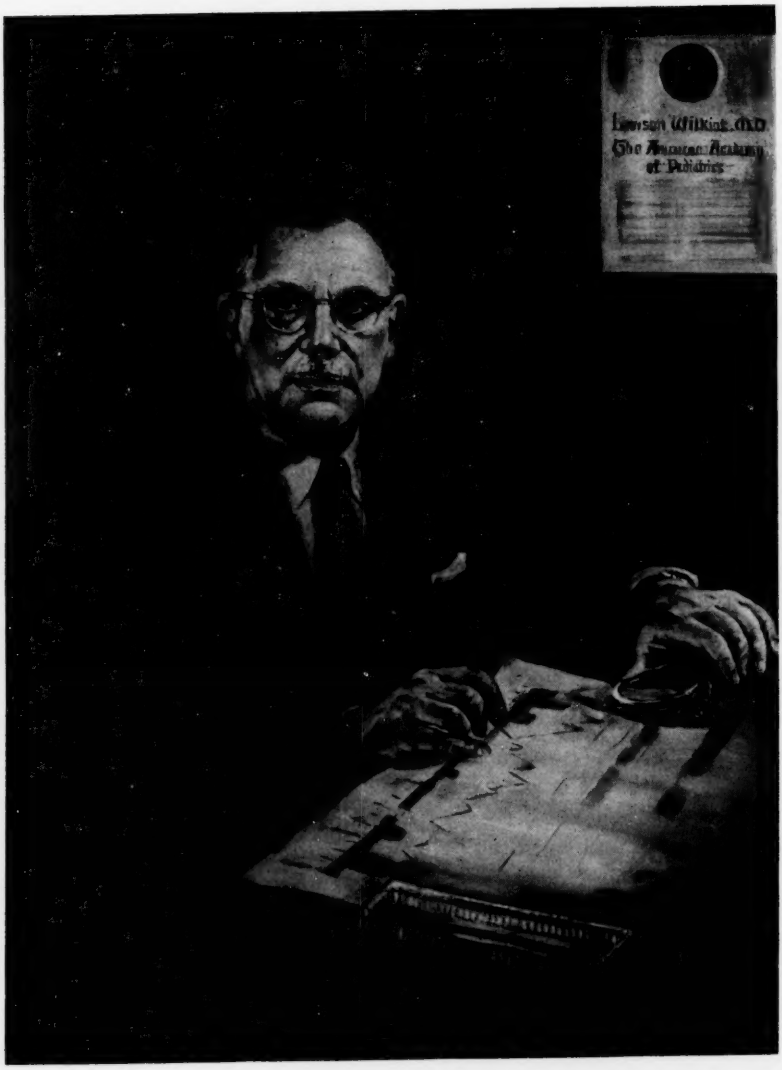
As a former President of this Society, you know from how deep within us this tribute comes. For in your long but seemingly unwearying years of endocrinological study and teaching you have reached us all.

None of us can fail to be informed and stimulated by your eminent studies. Whatever order and system the pediatric endocrinologist now has he owes to your original development of correlated clinical and biochemical analysis in this complex and, at times, mystifying field. Your characterization of the various patterns of thyroid deficiency in children opened the way to our current knowledge and management of this disabling, yet reversible disease process. Your incisive study of the disturbed physiology of the adrenogenital syndrome led you to a comprehension of the several varieties of this affliction which in turn has provided its victims with one of the most effective therapies known to our specialty. Your keen appreciation of fundamental biological relationships resting on a firm foundation of embryological and anatomical knowledge has enlightened our entire view of the nature of the disorders of gonadal development and function which blight the body and spirit of so many unfortunate children.

And with all these monumental achievements you have brought to your patients the comfort and assurance that can only be generated by the kindly concern of one who is genuinely devoted to others. And this same benevolence and interest reflects itself in the fine array of that highly select and yet numerous band of younger men and women who have been privileged to call you their teacher. Yet all of us interested in endocrinology through your explicit and instructive writings have shared in this privilege.

Hence it is only fitting that we here collectively, through your own Endocrine Society, tender you this small, but to us precious indication of our great indebtedness to you and of our ever constant affection for you—Doctor Lawson Wilkins!

THE KOCH MEDAL



DR. LAWSON WILKINS

THE AYERST FELLOWSHIP

DR. JOHN MAXWELL MCKENZIE

Dr. John Maxwell McKenzie is the recipient of the Ayerst Fellowship for 1961, presented by The Endocrine Society in acknowledgment of his contributions to our understanding of the pathogenesis of Graves' disease. Dr. McKenzie was born in Glasgow, Scotland, on November 13, 1927. His early schooling and University training were all in his native Scotland. He was Dux Medallist upon completing his secondary school training at Rothesday Academy. He received the M.B., Ch.B., from the University of St. Andrews, Dundee, in 1950. Dr. McKenzie's post-graduate training included: House Surgeon, house physician, Maryfield Hospital, Dundee; Assistant, Department of Pharmacology and Therapeutics, University of St. Andrews; Registrar, Maryfield Hospital. It was during this early phase of his training that he came under the influence of Professor R. B. Hunter whose enthusiasm for research sparked in Max McKenzie the desire to win a place for himself in Academic Medicine. With this in view he joined Dr. E. B. Astwood's laboratory as a Research Fellow in Endocrinology, New England Center Hospital, and Assistant in Medicine, Tufts University Medical School. He returned to the Maryfield Hospital, Dundee, as a Registrar for a one year period and once more joined Dr. E. B. Astwood's department as a Research Associate in Medicine, New England Center Hospital and an Instructor in Medicine, Tufts University Medical School. It was during this time that his thesis for the degree of M.D. of the University of St. Andrews entitled "The Bioassay of Thyrotropin in Serum" was accepted and awarded Honours and the University Gold Medal. In 1959 he joined the Endocrine-Metabolic Unit of the Department of Medicine and the McGill University Clinic, Royal Victoria Hospital, Montreal. At present he is in charge of the Thyroid Laboratory, a Lecturer in Medicine, McGill University and a Clinical Assistant in the Department of Medicine, Royal Victoria Hospital.

Dr. McKenzie's contributions to endocrinology have been in the field of thyroid physiology, particularly in the development of bio-assay techniques for T.S.H. and the "thyroid activator" of hyperthyroidism. It is with the isolation and characterization of the latter factor that he is particularly concerned at the present time. The Ayerst Fellowship will allow him to spend time in the laboratories of Dr. C. J. O. Morris at the London

Hospital, London, England, gaining further experience in techniques of separation of protein and polypeptide hormones.

Dr. McKenzie is a careful and able investigator, a competent physician, a quiet, amiable and humble colleague. He succeeds in infusing a unique enthusiasm into all those with whom he comes in contact, an attribute which permits his laboratory to accomplish an impressive amount of productive work. The Awards Committee has every reason to predict that his future contributions will continue to reflect credit upon this Society.

THE CIBA AWARD

DR. ERNST KNOBIL

The 1961 recipient of the Ciba Award is *Dr. Ernst Knobil,* Assistant Professor of Physiology in the Harvard Medical School. The Endocrine Society takes pride in adding his name to the roster of distinguished men and women upon whom this award has been conferred.

Dr. Knobil was born in Berlin and reared in Paris, where he was schooled in the demanding French educational clime. Although he attended the Claude Bernard Lycée, it is not likely that this circumstance launched his career in experimental physiology in that it was not until sometime later that he learned about Claude Bernard! He was early inclined to the arts and once contemplated a career in architecture. A youthful fling at sculpturing at a New York art school yielded some objects for exhibit, but their present fate is obscure.

At age 15 Ernie entered the School of Agriculture at Cornell to major in animal husbandry. Art and architecture paled before the spectacle of rural America, which inspired him to indulge in the intricacies of wool growing, herd fattening and flock culling. That his perceptive powers were challenged is evident from the fact that he made the Livestock Judging Team and won a number of prizes.

After the sophomore year he was called to the military and assigned to the Information and Education Branch at Fort Dix as a specialist. He came to head the Indoctrination staff and with typical individual initiative he voluntarily taught French and German courses by night for officers going overseas and established a training course for information specialists. Contact with outside education came in the form of an assignment to arrange for the schooling of the children of camp personnel in neighboring public schools. Convincing local school boards to accept more

students into schools already overcrowded and understaffed sharpened his persuasive powers.

After the war Dr. Knobil returned to Cornell, bent on a closer look at the sciences. His enthusiasm for the pure sciences was fired by distinguished scholars and teachers such as Sidney Asdell, Glen Salisbury and Samuel Leonard. It was Asdell's course "The Physiology of Reproduction" that settled the direction of his graduate work, which was done in zoology with Dr. Leonard. Courses with J. B. Sumner and Donald Griffin among other campus notables deepened his scientific curiosity.

He gained his postdoctoral experience in research at Harvard with R. O. Greep and associates. With Greep he spearheaded the demonstration of the species specificity of growth hormone in primates and thereby provided the clue to its successful use in man. He has independently made important observations in the fields of hypophyseal, adrenal and reproductive physiology. Currently, as evidenced by his report at this meeting, he is exploring avenues in cellular biology looking to the mechanism of hormone action at the molecular level. More than a talented investigator, Dr. Knobil is a stimulating teacher and has regularly enjoyed the acclamation of students for his orderly and dynamic lectures. He is a Markle Scholar and a member of the Editorial Board of *Endocrinology* and the *American Journal of Physiology*. As Acting Editor of *Endocrinology*, for one-half year he discharged the responsibilities of that office with skill and resourcefulness.

The fates of fortune have surrounded this young scholar. Within the month he will, at thirty-four years of age and as Professor and Chairman of the Department of Physiology, occupy the Mellon Chair in the School of Medicine at the University of Pittsburgh.

Dr. Knobil, the distinguished recognition which comes to you here as recipient of the 1961 Ciba Award is based on your outstanding achievements in science. It is in keeping with the important responsibilities in medical education and research which lie before you. You have the congratulations and good wishes of this Society.

THE UPJOHN SCHOLARS

The Upjohn Scholars for 1961 are *Dr. and Mrs. Wilhelmi.*

Dr. Jane A. Russell (Mrs. Wilhelmi) was born in Los Angeles. She received her B.A. and Ph.D. degrees from the University of California and spent a year of post-doctoral study at the Institute for Experimental Biology at Berkeley. In 1938 she moved to Yale to continue her work on the pituitary. It was while at Yale that she married Alfred Wilhelmi. In 1950 the Wilhelmis moved to Emory University where Dr. Russell holds the position of Associate Professor of Physiological Chemistry.

DR. JANE A. RUSSELL

DR. ALFRED E. WILHELMI

In recognition of her classic studies on the role of the adenohypophysis in carbohydrate and protein metabolism, Dr. Russell was given the Ciba Award of the Endocrine Society in 1945. She is a member of the National Science Board, a high honor indeed, for this body of scientists appointed by the President of the United States is charged with the responsibility of guiding the policies and operations of the National Science Foundation.

Dr. Alfred E. Wilhelmi was born in Lakewood, Ohio. Following receipt of his B.A. degree from Western Reserve University, he spent three years at Oxford University as a Rhodes Scholar. From Oxford he went to Yale, Department of Physiological Chemistry, where he rose to the rank of Associate Professor. In 1950 he became the Chairman and Professor, Department of Biochemistry, Emory University.

Dr. Alfred Wilhelmi has made notable contributions in a number of areas including creatine metabolism, parathyroid hormone, growth hormone and the comparative endocrinology of pituitary hormones. As a member of the Endocrinology Panel of the National Institutes of Health, he has made a most notable contribution to endocrinology by making available high purified pituitary hormones for metabolic studies. Many of his fellow endocrinologists are most grateful indeed.

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PRESENTATION OF CERTIFICATE OF MERIT OF THE ENDOCRINE SOCIETY

Henry H. Turner, The Endocrine Society has thought of you so often for so many years and been so grateful to you that it seems about time to put some tracing of this regard down on formal paper. We celebrate this occasion here tonight. Physician since 1921, you have practiced endo-

crinology from the difficult days when tools were poor, to the more abundant present when tools are better. Among other contributions, an account of yours in 1938 brought attention to a syndrome which has had incessant study and which now serves to show more of the essentials of man's organization than was thought comprehensible even five years ago. Teacher since 1924 at the University of Oklahoma and wherever else our Society and many others have called on you, you have instructed in the necessary examination of disease. Citizen since birth, you have been a willing participant in the work of the world, both at home in Oklahoma City and outside, wherever affairs have taken you. Secretary-Treasurer of The Endocrine Society since 1941, by a genius for arrangements you have held us all together. And so we come to this Certificate of Merit of The Endocrine Society. Because you have been a good fellow and enjoyed all of this hard work mightily, we shall, in our thoughts, tie this roll of parchment lightly in a gay ribbon as we wish you well, and add a cheer to all the solemn words.

The 1962 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors. Endocrine Society awards are limited to citizens of the United States and Canada.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle and in 1960 was presented to Dr. Emil Witschi. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954.

The Ciba Award

The Ciba Award is given in recognition of the meritorious accomplishments of an investigator in the field of basic or clinical endocrinology who has not reached his thirty-sixth birthday before June 1 of the year in which the Award is presented. This Award was established in 1942 and first presented in 1944. The Award is presently in the amount of $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $7,500, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

Thirteenth Postgraduate Assembly in Endocrinology and Metabolism

Under the Co-Sponsorship of The Endocrine Society
and The National Institutes of Health
Bethesda, Maryland
October 2–6, 1961

A comprehensive review of clinical endocrine problems and current research activity in these areas will be presented. For further information, write to: Dr. Roy Hertz, National Institutes of Health, Building 10, Bethesda 14, Maryland. The fee will be $100.00 for physicians, with a reduction to $30.00 for Residents and Fellows. Enrollment limited to 100.

Acknowledgment

The Editorial Board of The Journal of Clinical Endocrinology and Metabolism wishes to acknowledge with gratitude the generous and expert aid of the following reviewers:

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Symposium on Recent Developments in Research Methods and Instrumentation

October 9–12, 1961

NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MARYLAND

Instrument Symposium. Primary topics for discussion on the scientific program include: 1) Applied Gas Chromatography, 2) Factors Influencing Interpretation of Spectra, 3) Electron Magnetic Resonance, 4) Thermogravimetric Analysis, 5) Electron Probe Analysis, 6) Application of Physiological Instrumentation to Clinical Problems, and 7) Optical Rotatory Dispersion. Chairman: Dr. Julius Sendroy, Jr., Naval Medical Research Institute, Bethesda 14, Maryland. Executive Secretary: Mr. James B. Davis, National Institutes of Health, Bethesda 14, Maryland.

Research Equipment Exhibit. Examples of the latest types of research equipment will be exhibited by instrument manufacturers. Chairman: Mr. Louis Heiss, American Instrument Co., Inc., 8030 Georgia Avenue, Silver Spring, Maryland. Exhibit Manager: Mr. James B. Davis, National Institutes of Health, Bethesda 14, Maryland.

International Congress on Hormonal Steroids

MILAN, ITALY, *May 1962*

The scientific program includes: a) Six symposia at which lectures will be given by prominent invited speakers; (b) Sessions of short communications; c) Private scientific meetings and round table discussions.

The following 6 symposia are planned: 1) New steroids with hormone-like activities; 2) Pathways of synthesis and metabolism of hormonal steroids; 3) Control of synthesis and release of steroid hormones; 4) Mechanism of steroid action; 5) Substances which affect the synthesis and action of steroid hormones; 6) Non-hormonal activities of steroids.

Official languages of the Congress will be English, French and Italian. The Proceedings of the Meeting will be published in book form after the Congress.

Organizing Committee: Honorary President, Dr. Gregory Pincus, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, U.S.A. General Chairman, Prof. Emilio Trabucchi, Direttore, Istituto di Farmacologia e Terapia, Universita di Milano, Italy. Congress Secretaries, Prof. Dr. L. Martini and Prof. Dr. A. Pecile, Istituto di Farmacologia e Terapia, 21 Via A del Sarto, Milano, Italy.

For further information, application forms, and forms for submission of papers please write to either of the Secretaries.